1967

Organophosphorus Resistance In The Two-spotted Spider Mite Tetranychus Urticae Koch

Donald Hardesty Herne

CANADA

MICROFILMED

BY

CENTRAL MICROFILM UNIT

PUBLIC ARCHIVES

OF

CANADA

OTTAWA, ONTARIO

MICROFILMÉ

PAR LE

SERVICE CENTRAL DU MICROFILM

ARCHIVES PUBLIQUES

DU

CANADA

OTTAWA, ONTARIO

DATE Dec 20/67 REDUCTION 15

OPERATOR / OPERATEUR S Laughlin EXPOSURE / EXPOSITION 81

ARC-178(1/66)

CANADA

NATIONAL LIBRARY OF CANADA

CANADIAN THESES

ON MICROFILM

BIBLIOTHÈQUE NATIONALE DU CANADA

THÈSES CANADIENNES

SUR MICROFILM

NL-101(1/66)

ORGANOPHOSPHORUS RESISTANCE IN THE TWO-SPOTTED SPIDER MITE TETRANYCHUS URTICAE KOCH.

by

Donald H. C. Herne

Department of Zoology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Canada

August 1967

ABSTRACT

The inheritance of parathion-resistance and the patterns of cross-resistance were investigated in the Niagara stock of the two-spotted spider mite Tetranychus urticae Koch. Five strains were utilized: one susceptible, one parathion-resistant, and one partially-resistant, and the other two were susceptible and parathion-resistant strains selected with Bidrin. One susceptible substrain was reared from one of the original susceptible strain females.

Mites were exposed upside down on cellulose tape and sprayed with an acetone solution of the toxicant in a Potter Spray Tower. Mortality was much reduced at low post-treatment temperatures, but temperatures above 24°C increased the mortality. Mortality was decreased at very high relative humidities. The pre-treatment temperature had little effect, nor did post-treatment relative humidities close to 100%. Month-old adult females were much less susceptible than adults 2 - 4 days old. Mortalities differed appreciably according to the time of day when the test was conducted. The addition of either olive oil or corn oil to the acetone solvent increased the mortality.

Reciprocal crosses between parathion-resistant and the susceptible strains showed that the resistance was almost completely dominant, the results of reciprocal crosses being identical. Backcrosses of the

F_1 female hybrids with susceptible males gave offspring clearly separated into two types in a 1:1 ratio, proving that the character was monofactorial. Tests proved that the susceptible strain was essentially pure for susceptibility. The resistant strain was no less fecund or heat-hardy than the susceptible strain, and showed a significantly higher egg viability.

Incubation of homogenates in vitro with C^{14}-malathion showed that the parathion-resistant strain detoxified almost twice as much malathion as the susceptible strain. Most of the hydrolysis took place at the carboxyester bond but there was also appreciable phosphatase activity. The activity and malaoxon-sensitivity of the cholinesterase (ChE) enzymes in the resistant strain was similar to that in the susceptible strain.

The cross-resistance of the parathion-resistant strain to other OP compounds ranged from 2-fold to phosphamidon and Bidrin, up in ascending order through malathion, fenthion, demeton, mevinphos and ethion, to 1000-fold to dimethoate. The highest levels of cross-resistance were to those compounds which were most toxic to the susceptible strain, namely those with a carbomethoxy group (mevinphos) and the phosphorodithioates (ethion and dimethoate). However, there was no consistent difference in cross-resistance between the phosphates, phosphorothioates or phosphorodithioates. Although cross-resistance was generally less with methyl than with ethyl compounds, there was no consistent difference between the two groups. No cross-resistance was shown to the chlorinated acaricide dicofol, and only a slight cross-resistance to the carbamate Temik.

When the susceptible strain was selected with Bidrin for 10 generations it developed as little resistance to Bidrin and as high a resistance to parathion as the parathion-selected resistant strain; the cross-resistance levels to phosphamidon were identical in both strains. Selection of the parathion-resistant strain with Bidrin did not increase either the resistance to Bidrin or to parathion.

Compounds to which the OP-resistant strain showed the least cross-resistance, namely the carbamoyl-oxime phosphates, Bidrin and phosphamidon, and the carbamate Temik, offer the promise for control of T. urticae and perhaps other tetranychids in which the resistance mechanism is detoxication.

ACKNOWLEDGEMENTS

I express my gratitude and sincere thanks to Professor A. W. A. Brown, Head of the Department of Zoology whose interest, instruction, guidance, and encouragement were responsible for the initiation and completion of this work.

I am indebted to the Canada Department of Agriculture for granting the leave of absence necessary to attend the graduate school and for providing the facilities to complete much of this work.

I am very grateful to those members of the Research Station, Canada Department of Agriculture, Vineland Station who helped in any way during this investigation and especially the following: Dr. D. A. Chant, former director, and Dr. W. B. Mountain, present director, who co-operated in every way to make this work possible; Mr. W. L. Putman, and Dr. J. H. H. Phillips whose encouragement and interest were invaluable; Mr. W. Cairns, former Student Assistant, Mr. M. Howard, former Technician, Mr. Lund, Assistant Technician, for their able assistance; Mrs. M. Mountney, and Mrs. M. Bowring for typing portions of the rough draft.

Mrs. P. Morse, of the Statistical Research Service, Canada Department of Agriculture, Ottawa, kindly advised on

statistical procedures and arranged for computer probit analysis of much of the data. I am very grateful to Mr. B. Stone and Mr. Meir Ziv, graduate students of the Department of Zoology for their assistance and guidance in the enzymatic study.

I am grateful to Mr. Stan Pluzak, Mr. A. Gage and other staff members of the Department of Zoology who in many ways aided in this work.

Niagara strains of T. urticae were kindly supplied by Dr. Aller, Niagara Brand Chemicals, F. M. C. Middleport, N. Y. I also thank the personnel of the Bio-Graphic Unit, Canada Department of Agriculture, Ottawa, for their assistance with much of the art work.

The agricultural chemical companies, listed under material and methods, kindly supplied a number of the compounds used in this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Phytophagous mites of the family Tetranychidae, Order Acarina, are now considered the most important pests of fruit, field, and greenhouse crops throughout the world (Glass 1960, Jeppson and Jesser 1962, Hill 1965, Helle 1965a). The two-spotted spider mite Tetranychus urticae Koch (= telarius auctt.) is one of the most injurious species because of its wide distribution and host range. The control of this species is hindered, like that of other tetranychids, by its quick development of resistance to organophosphorus (OP) compounds and other acaricides. Although the ability to develop resistance is fairly general throughout the arthropods (Brown, 1961a), tetranychid mites appear to be unique in the rapidity with which they develop resistance to this group of compounds (March 1959, Helle 1965a).

It is generally agreed that the inheritance of OP-resistance in tetranychids is based mainly on a single dominant gene transmitted by both sexes (Taylor and Smith 1956, Andres and Prout 1960, Helle 1965a, Schulten 1966); however, one investigator has reported that the inheritance is polygenic, involving dominant semi-lethal genes and a recessive factor (Dittrich, 1963a). Two mechanisms of OP-resistance are known in T. urticae. One, found in the Leverkusen strain from Germany, is a reduction in the sensitivity of cholinesterase (ChE) to inhibition by the OP compound; the other, found in the Blauvelt strain at Cornell

University, is an increased detoxication of the OP compound before it can inactivate the ChE in the mites (Voss and Matsumura, 1964).

A serious mite control problem first arose in Ontario orchards and greenhouses a few years after the introduction of the first OP compounds, parathion and TEPP. Today, T. urticae and the European red mite Panonychus ulmi (Koch) are generally resistant to most of the OP compounds applied in Ontario greenhouses and orchards, including parathion, TEPP, malathion, azinphos-methyl, mevinphos, phosphamidon, demeton, and ethion (Putman and Herne 1959; Herne 1960, 1961a, and 1961b; Herne and Putman 1966). However, certain of these populations may be still controlled by one or other of the last-named compounds such as ethion.

It is therefore important to establish the degree to which a population, made resistant by selection with one OP compound, becomes cross-resistant to the others. This cross-resistance may extend to compounds in entirely different chemical groups, and the converse is also true.

Although a body of information has been obtained on levels of resistance and cross-resistance in mite population from field assessments and laboratory experiments, it is scattered through such a variety of strains, species and OP acaricides that no consistent pattern has emerged for any of the OP selecting agents. There is also little agreement as to how long various OP compounds will remain effective before economic or maximum levels of resistance develop, or how quickly it returns after reversion from OP-resistance has occurred. It is also not known whether low levels of cross-resistance associated

with full resistance to the initial selecting agent can develop into high levels of resistance to the new selecting agent, or whether further selection with a different OP compound will change the cross-resistance pattern established by the initial selecting agent.

The general purpose of this investigation was to provide precise knowledge of the development of OP-resistance and of patterns of cross-resistance in a single species and strain of tetranychid mite. This was the Niagara strain of Tetranychus urticae, commonly used for screening new OP compounds for commercial development. Parathion-resistant and normal susceptible substrains were compared, and the following characteristics of OP-resistance were determined:- a) the mode of inheritance of the resistance using parathion as the test insecticide, b) the type of resistance mechanism, whether an insensitive cholinesterase or increased detoxication, c) the cross-resistance pattern from parathion to various OP compounds. From the results of these studies, the relationships between structure and toxicity and between structure and resistance hazard have been examined with a view to discovering OP compounds which remain potent against not only a susceptible but also a parathion-resistant strain.

In order to carry out the above investigations it was necessary to develop an accurate toxicological test method, and to determine how its accuracy was influenced by such factors as temperature and the age of the mites. It was also essential to determine whether the parathion-resistant strain differed from the normal in bionomic characteristics such as egg-viability and heat-hardiness.

LITERATURE REVIEW

Resistance may be defined as the loss of susceptibility of an arthropod population to a pesticide which originally controlled it. Resistance is therefore a characteristic of certain populations within a susceptible species (Brown, 1963a). It has been described as the formation of a new physiological race by directed selection, in which survivors of a pesticide pass on inborn resistance to their progeny. There is little or no evidence for postadaptive resistance since resistance can develop in a population only if the resistance alleles are present in its gene pool at the time of selection (Crow, 1957).

There are many excellent reviews of the resistance problem. Those which emphasize genetic studies include those by Busvine (1960), Milani (1960), Georghiou (1965) and Oppenoorth (1965), whereas those by Winteringham (1962) emphasize the physiological aspects of resistance. Brown (1958, 1960 a,b, 1961 a,b, 1963 a,b,c) has exhaustively reviewed the world resistance problem with respect to the extent of resistance development, the genetics of resistance, and the biochemical mechanism of resistance in arthropods. Unterstenhöfer (1961) and recently Helle (1965a) have reviewed the literature relating to resistance in the Acarina with emphasis on tetranychid mites. Hansen et al. (1963) and Jeppson (1963) have reported their detailed cross-resistance studies in tetranychids.

Resistance is now the major problem in the chemical control of pest arthropods and has been reported to have developed in nearly 200 species (Brown, 1967 in press). Of these, no less than 39 species of insects and mites are known to be resistant to the OP compounds. The first reports of organophosphorus resistance in tetranychid mites, in the early 1950's, were from greenhouse and orchard areas of the U. S. A. (Garman 1950, Cutright 1956, Taylor and Smith 1956). Now OP-resistance is known in at least 12 species of mites including some predacious species, and the resistance in the important tetranychid species occurs in most regions of the world (Smith 1960; Unterstenhöfer 1960, 1961; Glass 1960; Brown 1961a; Helle 1965a).

Even although one compound may have selected the resistance alleles controlling a resistance mechanism in an arthropod species, it can confer cross-resistance to other compounds of a chemically similar group. Moreover the development of resistance within one group of similar compounds may induce a cross-tolerance to a chemically different group of compounds, although the converse may not be true. Cross-resistance therefore has led to serious control problems throughout the world (Brown, 1958).

Several pairs of compounds, however, have been found to which the cross-resistance is negatively correlated, so that selection for resistance to one compound increases the insects' susceptibility to the other (Brown 1961b) and Jeppson et al. (1964) reported finding negatively-correlated pairs of OP acaricides for Panonychus citri M^{c}G. The resistant P. citri strain had been field-selected, mostly with demeton, but several 2, 4, 5-trichlorophenyl phosphoramidate compounds

were found to be more toxic to this strain than to the susceptible one. However, Jeppson et al. (1965) later reported that the resistant strain had slowly developed resistance to these compounds.

To certain OP-compounds the degree of cross-resistance shown by OP-resistant mites is quite low and perhaps no more than a general vigor tolerance. For example, in some Ontario orchards parathion- and malathion-resistant P. ulmi can still be controlled by the OP compounds ethion (Herne, 1962). Azinphos-methyl was initially effective against OP-compound resistant mites in Ontario orchards, but in this case a single season of use resulted in its becoming ineffective for reasons unknown (Herne, 1961a).

Organophosphorus-resistance in mites usually increases slowly at first and then accelerates rapidly (Unterstenhöfer, 1961). Resistance to parathion was developed faster by T. urticae when the selection pressure was high, only one severe selection being sufficient to produce a highly resistant strain in the laboratory but the ultimate resistance level attained is independent of the degree of selection pressure (Watson and Naegele, 1960). Jeppson et al. (1965) found that in P. citri resistance to Dowco 133 developed very slowly in the laboratory but very quickly in the field. The rate of increase may depend on the stage of the mite treated (Jeppson and Jesser, 1962) and the insecticide previously used (Hansen 1958, Jeppson et al. 1958, Watson and Naegele 1960).

Mite populations vary also in the rate at which they revert to susceptibility after organophosphorus treatment is discontinued. Garman (1950) in field experiments with P. ulmi, and Saba (1961a) and

Dittrich (1961) in laboratory experiments with T. urticae, reported the strains lost their resistance rapidly as soon as the treatments with parathion and other OP compounds were discontinued, whereas several investigators have reported no measurable reversion for long periods of more than 5 years after selection pressure was removed (Unterstenhöfer 1961, Helle 1965b). Evidently if the strain is genetically pure for the resistance factors there will be negligible reversion towards susceptibility (Helle, 1965a).

When the cross-resistance spectra of tetranychid mites, made resistant by selection with one or more OP compounds are studied in detail, usually they are found to extend to other OP compounds but not to non-phosphates such as the chlorinated acaricides (Andres and Reynolds 1958, Whitnall 1962, Unterstenhöfer 1961, Jeppson 1963). In most Ontario greenhouses and orchards where parathion had usually been the original selecting agent, T. urticae and the European red mite (Panonychus ulmi Koch) are resistant to varying degrees to the organophosphorus materials parathion, malathion, azinphos-methyl, mevinphos, phosphamidon, TEPP, and demeton (Herne, 1962). These OP-resistant mites could initially be effectively controlled with chlorinated acaricides, but now resistance to dicofol and ovex has appeared in a few orchards (Foott 1960, Herne 1961a). Helle (1959) found that populations of T. urticae and T. cinnabarinus Boisduval which had been selected mainly with parathion were cross-resistant to 8 OP compounds which had not been used previously; these included mevinphos, Thiometon, and Phenkapton. The mites were susceptible to the chlorinated acaricides ethoxyquin and dicofol. A strain of

P. citri, resistant to demeton, was found to be cross-resistant to 14 other organophosphorus materials (Jeppson et al. 1958). A Norwegian strain of T. urticae selected with parathion (Fjelddalen and Stenseth 1962) was found to be cross-resistant to the OP compounds diazinon, oxydemetonmethyl (methyl demeton), malathion, Phenkapton, phosphamidon, Thiometon and sulfotepp, but to retain its normal susceptibility to the chlorinated acaricide dicofol.

Detailed studies on cross-resistance in T. urticae have been made in the laboratory by Watson (1956), Hansen (1958), Bravenboer (1959), and Hansen et al. (1963). Hansen et al. (1963) worked with a "wild population" that initially showed a low level of resistance to parathion and a slightly higher resistance to malathion and oxydemeton-methyl (methyl demeton). Selection with parathion resulted in high levels of induced cross-resistance to sulfotepp, malathion, and oxydemetonmethyl (methyl demeton). Malathion was found to be a stronger selecting agent than parathion, conferring very high levels of resistance to parathion and oxydemetonmethyl, and moreover inducing a measurable cross-resistance to the chlorinated acaricide dicofol. The only other case of cross-resistance from OP compounds to chlorinated acaricides in T. urticae was reported by Bravenboer (1959), who found that a strain on peach that had a 20-fold parathion resistance with no cross-resistance to other OP compounds showed a 12-fold cross-resistance to dicofol. In another detailed cross-resistance study with T. pacificus and P. citri, Jeppson (1963) found no cross-resistance to chlorinated acaricides in strains selected by demeton plus parathion, but in contrast he confirmed that selection by

chlorinated acaricides gave high cross-resistance to OP compounds. This is the opposite to what occurs in insects, in which selection with chlorinated insecticides does not give cross-resistance to OP compounds whereas selection with OP compounds confers high levels of cross-resistance to DDT and other chlorinated insecticides (March 1959, Brown 1961a).

Jeppson (1963) noted that the two different mite species he studied showed discrepancies as well as similarities in their cross-resistance patterns within the OP compounds. Although some trends have been discovered in the cross-resistance patterns of OP-resistant strains, Helle (1965a) has been forced to state that "it is still impossible to get a clear picture". He cites the following reasons for this situation: (i) the lack of standardized and precise test methods; (ii) misinterpretation of data because the tests included too few test dosages; (iii) the variation of expression of OP-resistance depending on the temperature; (iv) incomplete knowledge of the past history of test populations with respect to insecticide applied; and (v) the inadequacy of field resistance assessments to include all the pertinent OP compounds. One set of detailed cross-resistance studies, that of Watson (1956) with T. urticae, was unfortunately marred by the development of resistance in his control strain. Inter-strain differences and peculiarities in the cross-resistance pattern are to be expected in mites as they have been observed in insects. However, the pattern of OP-resistance in 6 different populations of houseflies in California, where one strain was highly diazinon-resistant but only slightly malathion-resistant and the other the reverse, was found by

Georghiou and Bowen (1966) to reflect the different past histories of chemical control practices.

The organophosphorus compounds have been classified as ganglionic nerve poisons in arthropods (Brown 1963b). Their effect on the ganglion causes a single presynaptic spike to be multiplied into continuous postsynaptic discharge and this facilitation is subsequently followed by synaptic block. The toxic effect of OP compounds is proportional to their anti-cholinesterase activity. The cholinesterase (ChE) molecule is phosphorylated by an OP compound at the esteratic site whose normal function is to accept the substrate acetylcholine (ACh). Once phosphorylated, the ChE enzyme is inhibited in its role of destroying the ACh normally liberated at a nerve synapse to mediate the transfer of an impulse across a synapse. The accumulated ACh eventually blocks the synapse and causes paralysis.

Most organophosphorus insecticides are esters of phosphorothioic acid or phosphorodithioic acid characterized by a P=S group. In tissues of mites, as in those of insects, mammals and plants, the P=S linkage is oxidized to the P=O linkage to form a potent ChE inhibitor. Since carbamate compounds are also inhibitors of the ChE enzyme, it is not surprising that cross-resistance occurs between these groups of compounds.

The resistance mechanisms developed by resistant strains of insects and mites have been described for the 3 main types of resistance including OP-resistance (Brown 1958, 1963c; Perry 1964). The resistance mechanism, for the chlorinated or otherwise non-phosphate acaricides, have not as yet been characterized (March 1958).

Resistance mechanisms have, however, been classified under three headings: physiological resistance involving chemical mechanisms enabling an arthropod to resist a poison that has entered its body; morphological resistance which prevents or retards penetration of a poison; and behavioural resistance involving an avoidance response to the poison (Brown 1958, Unterstenhöfer 1960, Winteringham 1962, Georghiou 1965, Jarczyk 1966).

All types may be involved in the resistance of a resistant individual or strain, but usually one predominates. Matsumura and Brown (1961) found that reduced penetration was a major mechanism in a strain Aedes aegypti resistant to malathion and DDT; absorption and retention of malathion was only 1/12 of the normal, whereas, on the other hand, there was no increase in the rate of detoxication of malathion. However, usually the major mechanism in OP-resistance is hydrolytic detoxication by means of esterase enzymes. These detoxifying enzymes arise in houseflies from genetic transformation of the aliesterases which normally hydrolyse aliphatic esters. They may be either phosphatases to hydrolyse parathion, paraoxon or other OP compounds, or they may be carboxyesterases to hydrolyse malathion. For example, van Asperen (1964) found that the normal aliesterases of houseflies are irreversibly inhibited by OP compounds, but that the modified OP-degrading esterases can be dephosphorylated and simultaneously hydrolyse the inhibitor. Three different OP hydrolysing enzymes were detected: those which degrade diethyl organophosphates such as paraoxon and diazinon and related diethyl compounds; those degrading both diethyl and dimethyl organophosphates; and those that attack dimethyl

organophosphates and malaoxon (van Asperen and Oppenoorth, 1960).

In contrast Jarczyk (1966) reported finding two OP-degradation esterases in houseflies and Lepidoptera, which he termed P=O and P=S phosphotriester hydrolases. These enzymes, however, were not specific for dimethyl and diethyl esters of phosphoric acid since they also degraded thiophosphoric and thiophosphonic esters but at a lower rate. The P=O hydrolase was more effective in detoxication than the P=S enzyme. He traced the detoxication of fenitrothion (sumithion and Folithion) an analogue of parathion, to its final consection, as follows: fenitrothion is oxidized to its P=O ester; and both compounds are degraded by the two phosphotriester hydrolases so that if hydrolysis exceeds oxidation the compound is detoxified; the breakdown products are dimethyl thiophosphoric acid and dimethyl phosphoric acid as one moiety and nitrophenol as the other; the nitrophenyl moiety is reduced to aminophenol and then conjugated with D-glucose then to form non-toxic β-glucoside; the dimethyl phosphoric and thiophosphoric acids are hydrolysed by phosphatases and the products may be utilized in cellular metabolism.

The physiological mechanism of OP-resistance in mites was not studied until the 1960's when already much was known of the mechanism in houseflies. The demonstration ChE in spider mites was first reported by Voss (1960). In T. urticae McEnroe (1960) discovered that the ChE was mainly located in the central nervous system, while Mehrotra (1961) reported the occurrence of malic dehydrogenase in this mite. Dauterman and Mehrotra (1963) found that the ChE of T. urticae had no clear pattern of specificity to a number of esters in contrast

to that of houseflies which did show specificity for some esters. Biochemical resistance studies in mites have hitherto been restricted to T. urticae, since other species are so difficult to rear and handle.

Two mechanisms of resistance to organophosphorus compounds and carbamates have been discovered in T. urticae. In one OP-resistant strain, 'Blauvelt', the resistance mechanism is detoxication (Voss and Matsumura 1964, and Matsumura and Voss 1964), whereas in another strain, 'Leverkusen', the resistance mechanism is a cholinesterase enzyme insensitive to inhibition by OP compounds and carbamates (Smissaert, 1964). The latter mechanism was confirmed by Voss and Matsumura (1964), who found the ChE of the resistant Leverkusen SP strain to be 100-1000 times less sensitive than normal to OP inhibition and 6.5 times less sensitive than normal to inhibition by the carbamate insecticide carbaryl. Although the interstrain difference in the ChE insensitivity was less than reported by Smissaert, this could be attributed to the differences in methods employed, so that the authors could still conclude that the ChE of the resistant strain was "unusually insensitive to paraoxon".

This resistance involving an insensitive target site provides confirmation for the mode of action of OP compounds being ChE inhibitors. Such an OP-resistance mechanism does not occur in insects (Perry, 1964), but it has been recently discovered in an OP-resistant strain of the cattle tick, Boophilus microplus Canestrini. The resistant strain was found to possess a ChE insensitive to inhibition by paraoxon, coroxon, dichlorvos, diazinon and a P=O analogue of carbophenothion, and also insensitive to inhibition by the carbamate carbaryl. It also contained a ChE as sensitive as that present in the normal

susceptible strain, but this could have been due to a portion of the resistant strain being heterozygotes and thus possessing both the resistant and the normal ChE enzyme in the same individual. Smissaert (1964) found only the diazinon-insensitive type of ChE in his fully-resistant Leverkusen strain of T. urticae, but discovered both the sensitive and insensitive types of ChE to be present in the F_1 hybrids obtained by crossing the resistant mites and susceptible ones. The resistant Boophilus microplus degraded organophosphorus compounds at the same rate as the susceptible ones, and thus it was evident that increased detoxication was not a resistance mechanism in these ticks (Lee and Batham, 1966).

In the Leverkusen strain of T. urticae, however, there is some enhanced detoxication in the resistant strain (Matsumura and Voss 1964, Smissaert 1964). In initial studies Voss (1962) assayed egg homogenates of susceptible and resistant T. urticae with α-naphthylacetate as substrate and had found a lower aliesterase activity in the resistant homogenates. To ascertain whether aliesterases might have been transformed to OP-detoxifying esterases, Smissaert (1964) tested the homogenates of the Leverkusen R and S strains on α-naphthylacetate as a representative aliesterase substrate. He found the activity of the R homogenates to be 20 per cent less than that of the S homogenates on this substrate. He could detect at least three esterases in the R and S homogenates, but none were considered by him to have hydrolytic activity to diazoxon. Smissaert (1964) concluded by stating "there is little to support the hypothesis that the small differences in total activity between S- and R-homogenates is directly connected with the

mechanism of resistance". Matsumura and Voss (1964) confirmed that homogenates of the Blauvelt resistant strain had a significant but slightly lower hydrolytic activity against α-naphthylacetate than the Niagara susceptible strain. On the other hand the Blauvelt R showed higher activity than the Niagara S on other carboxyester substrates, especially β-naphthylbenzoate. In short, there was no general correlation between esterase activity with OP-resistance in the Blauvelt strain. The general conclusion may be made from the slight differences observed that aliesterase conversion is not responsible for the development of major degradation enzymes in organophosphorus-resistant mites.

Matsumura and Voss (1964) studied the two resistance mechanisms in the Blauvelt and Leverkusen resistant strains of T. urticae. They found that the resistant Blauvelt strain had a superior ability to break down parathion, malathion and malaoxon as compared to the Niagara susceptible strain. Four-fifths of the hydrolysis of the malaoxon molecule took place at the carboxyester bond, while there was also a substantial increase in phosphatase activity. The Leverkusen resistant strain on the other hand showed an inferior detoxicating ability as compared to the Niagara susceptible strain, although it was superior to that in the Leverkusen susceptible strain from which it had been developed by selection. There were only slight differences in the total uptake of malathion between any of the strains. Only the Blauvelt strain accumulated significantly less paraoxon than the Niagara strain, and the second Leverkusen strain (RR) also showed a lower malaoxon degradation rate than the Niagara susceptible strain.

In all studies of the Blauvelt resistant strain the Niagara susceptible has been the control strain, and yet differences in these strains have been emphasized that do not appear to relate to the resistance mechanism. In Niagara mites the rate of ChE inhibition in vivo by malathion was much higher and this was attributed to inter-strain differences in the levels of malaoxon (Matsumura and Voss, 1964). The lower rate of hydrolysis of β-naphthylbenzoate and higher rate of hydrolysis of α-naphthylacetate has already been mentioned, indicating slight differences in esterase activity between the strains.

Because of the importance of carboxyesterases as OP degradation enzymes in the Blauvelt resistant strain Matsumura and Voss (1965) partially purified them and compared them with those in the susceptible Niagara strain. Previously Matsumura and Brown (1963) had found that the carboxyesterase enzyme in malathion-resistant Culex tarsalis was not only 13 times more abundant than in the susceptible mosquitoes, but also was more heat-labile, had a lower dephosphorylation constant and turnover number and its pH optimum at 8.0 was narrower. Matsumura and Voss found that the malathion carboxyesterase in resistant T. urticae had a superior ability to hydrolyse malathion at the carboxyester as well as the phosphoroester bond. It had 20 times higher affinity toward malathion than the enzyme in susceptible Niagara mites, and was more sensitive to malaoxon inhibition. It may be identical to a part of the aliesterase which had been shown to hydrolyse β-naphthylbenzoate at a higher rate in the resistance strain. There was evidence that it was composed of two components, one DFP-sensitive and the other not. The malathion carboxyesterase in

T. urticae belongs to a different enzyme group from the malathion and parathion phosphatases.

Detailed studies by Voss and Matsumura (1965) showed similarities as well as differences between the cholinesterases (ChE's) in the resistant and susceptible Leverkusen strains. The maximum velocity (V_{max}), and the thermosensitivity of the enzymes did not differ significantly. These T. urticae cholinesterases were both unusual in that they hydrolysed propionylcholine faster than acetylcholine, the natural and more susceptible substrate in insects. The resistant enzyme differed from the susceptible in showing only 1/20th of the affinity for paraoxon, indicating an abnormally weak esteratic site. An even greater interstrain difference in hydrolytic rate of the enzyme was shown with n-propionylcholine cholinester. When the ChE's of the two strains were tested for their sensitivity to inhibition by four dialkyl analogues of malaoxon, the interstrain difference was greater when the dialkyl carbon chain length of the malaoxon substituent was least and the potency of the analogue was greatest. Thus with methyl substitution the interstrain difference was 1000-fold, but with butyl substitution it was 3-fold.

The ChE of mites, shown above to be different from that in insects, may have something in common with the pseudo-ChE of mammals since there is some evidence that it has a broader and less specific anionic site. But Voss and Matsumura (1965) concluded that mite ChE is a "true" cholinesterase with a slightly broader esteratic site and a very general anionic site as compared with the cholinesterase of insects and mammals. It is of interest that the Leverkusen R strain,

T. urticae belongs to a different enzyme group from the malathion and parathion phosphatases.

Detailed studies by Voss and Matsumura (1965) showed similarities as well as differences between the cholinesterases (ChE's) in the resistant and susceptible Leverkusen strains. The maximum velocity (V_{max}), and the thermosensitivity of the enzymes did not differ significantly. These T. urticae cholinesterases were both unusual in that they hydrolysed propionylcholine faster than acetylcholine, the natural and more susceptible substrate in insects. The resistant enzyme differed from the susceptible in showing only 1/20th of the affinity for paraoxon, indicating an abnormally weak esteratic site. An even greater interstrain difference in hydrolytic rate of the enzyme was shown with n-propionylcholine cholinester. When the ChE's of the two strains were tested for their sensitivity to inhibition by four dialkyl analogues of malaoxon, the interstrain difference was greater when the dialkyl carbon chain length of the malaoxon substituent was least and the potency of the analogue was greatest. Thus with methyl substitution the interstrain difference was 1000-fold, but with butyl substitution it was 3-fold.

The ChE of mites, shown above to be different from that in insects, may have something in common with the pseudo-ChE of mammals since there is some evidence that it has a broader and less specific anionic site. But Voss and Matsumura (1965) concluded that mite ChE is a "true" cholinesterase with a slightly broader esteratic site and a very general anionic site as compared with the cholinesterase of insects and mammals. It is of interest that the Leverkusen R strain,

characterized by the insensitive ChE, is resistant to the carbamoyl oxime Temik, reported to control most OP-resistant mite (Weiden et al. 1965). It did in fact have full potency against the Blauvelt R strain (Voss and Matsumura, 1964) thus confirming the difference in mechanism between the Leverkusen and the Blauvelt and most other OP-resistant strains.

A number of new organophosphorus compounds have been discovered to be effective against mites resistant to parathion, demeton and other commonly used OP compounds. Jeppson et al. (1965) found Dowco 133 (O-isopropyl O-2, 4, 5-trichlorophenyl phosphoramidothioate) and a similar phosphoramidate to show LC_{50} levels which were no higher with an ethion-selected resistant strain than with a susceptible strain of T. pacificus. However, appreciable resistance to Dowco 133 was developed by P. citri slowly in the laboratory and rather quickly in the field. These authors (1964) had previously reported that many of the phosphoramidate series of compounds with the 2, 4, 5-trichlorophenyl structure were slightly more toxic to demeton- and parathion-selected resistant strains of P. citri than to the normal strain. In contrast, a few analogues of the phosphoramidate series were more toxic to the susceptible strain than the resistant ones in both T. pacificus and P. citri.

Allen et al. (1964) reported that Bidrin, a carbamoyl phosphate, controlled a strain of T. urticae resistant both to OP compounds and to chlorinated acaricides, but their results indicated that phosphamidon was less effective. Azodrin, an analogue of Bidrin with one less methyl group, gave good control of OP-resistant T. urticae and P. ulmi

in Michigan (Howitt 1967, personal communication). The carbamate insecticides Dimetan (5:5-dimethyl-dehydro resorcinyl N-dimethylcarbamate) and Pyrolan (1-phenyl-3-methyl-pyrazolonyl N-dimethylcarbamate) were reported by Tew and Kirby (1953) to have contact and systemic acaricidal activity. It is interesting that when in the early 1950's the dimethylcarbamic group was replaced by diethyl thiophosphoric acid to give the OP ester diazinon, the result was an important insecticide but a rather poor acaricide.

Recently, Weiden et al. (1965) have reported on the O-methyl-carbamoyl oximes, "a new class of carbamate insecticides-acaricides"; among these, both Tranid (UC20047A) and Temik (UC21149) were as effective against the highly OP-resistant Cranbury strain of T. urticae as against the normal strain. The carbamoyl group appears to be linked with acaricidal activity and there is some evidence it is effective against mites generally very resistant to most OP compounds.

The effects of malathion analogues were studied in the Blauvelt strain of T. urticae, 60-fold resistant to malathion, and in the Niagara susceptible strain (Voss et al. 1964). The resistant strain showed a wide range of cross-resistance against the carboxyester substituents of malathion and malaoxon but the resistance factor diminished with an increase of the diphosphoroester group beyond the propyl chain length. The potency or inhibitory power increased as the chain length of the carboxyester substituents was increased. There was a linear relationship between toxicity of malathion, malaoxon, and dimethyl acethion analogues, and the resistance factor, obtained by comparing the LC_{50}'s of the susceptible and resistant strains, but the

relationship was not linear for oxydemetonmethyl (methyl demeton) or for the carbamate Temik®. The linear relationship was caused by a change in susceptibility of the susceptible strain only, and therefore the resistance factor need not indicate relative potency. The results indicate that with OP compounds, of a similar chemical nature, the greater the potency to a susceptible strain, the greater is the resistance factor that may develop. The conclusion was that with such similar compounds, as the malathion analogues, small modifications of the molecular structure are not likely to nullify OP-resistance in spider mites. But this may not be true with radically different OP compounds. Some OP compounds are still effective against OP-resistant P. ulmi as already mentioned. It is known that in insects, resistance to malathion, a dimethyl phosphate, is different from resistance to parathion and diazinon which are both diethyl phosphates (Brown, 1963a). A number of OP compounds with the phosphate group ($\overset{O}{\overset{\|}{P}}$-O), including mevinphos and phosphamidon, have a high inherent toxicity over a wide dosage range. The carbamoyl group appears to be linked with acaricidal toxicity whether or not it is part of an OP molecule. Menn and Stoffey (1965) found that out of 44 carbamoyloxy phosphorodithioate compounds tested against susceptible T. urticae, about 26 were highly toxic. Jeppson et al. (1966) evaluated 34 of these compounds for their effectiveness against OP-resistant T. pacificus developed by ethion selection, as well as OP-resistant P. citri developed by demeton selection, as compared to their effectiveness against the normal strains of each genera. Four dialkoxyphosphorodithioylethyl carbamates tested were found to be slightly toxic to both susceptible strains but

not toxic to the resistant ones. However, the addition of a CH_2S group to these compounds resulted in increased toxicity to both the S and R strains of each genera. A number of interspecies and intrastrain differences were found in the responses to the different compounds of the series. For example both strains of T. pacificus, and the R strain of P. citri were more susceptible to the diethoxy-P series of compounds than the S strain of P. citri. In general the R strain of T. pacificus was more susceptible than the R strain of P. citri to a series of compounds with different alkoxy-P moieties. A few compounds in each series tested had a very low resistance (3-fold to 5-fold) to one or other of the species but not to both. Jeppson (1965) felt confident that "there are, no doubt, toxicants to which mites cannot readily develop resistance". The answer may also lie in finding OP compounds to which mites can develop only low levels of resistance. Perry (1964) has concluded that OP compounds, unlike the chlorinated insecticides, induce resistances which can develop only to a certain level characteristic of the compound beyond which no further resistance can be selected.

The genetics of insecticide resistance in spider mites has been reviewed by Oppenoorth (1965) and more recently by Helle (1965a). Most of the studies have been concerned with the OP-resistance of T. urticae (Wood 1952, Taylor and Smith 1956, Watson 1956, Dittrich 1961 and 1963 a,b, Helle 1962, Schulten 1966). The inheritance of parathion-resistance in T. pacificus has been investigated by Andres and Prout (1960).

The genetics of resistance in tetranychid mites is unusual because

they are arrhenotokous, in that haploid males develop from unfertilized eggs whereas diploid females develop from fertilized eggs. Unfertilized females produce only males; fertilized females produce both females and males in a ratio of about 2:1. Also sib-mating is common since females tend to lay their full egg complement in a small area of one host leaf. The males moult to sexually-mature adults about 12 hours before the females, and remain on or near the quiescent deutonymph females until they moult to adults (Herne, 1957).

Helle (1965a) discussed the effect of arrhenotoky on the population genetics of tetranychids with respect to resistance and other factors. He concludes that recessive alleles cannot escape selection in males because they are haploid and hence homozygous for all genetic characters. Loss of favourable mutants through random drift will occur less frequently. As a consequence deleterious alleles under the selecting environmental conditions will be eliminated in a relatively few generations and characters favouring fitness will be quickly fixed. By contrast, although variability within populations is limited because of the tendency to homozygosity, interpopulational differences are liable to be established as a consequence of contemporary mutations and the establishment of new gene combinations. Helle (1965a) also indicates that the study on the inheritance of resistance by means of interstrain crosses may be hampered by incompatibility barriers which result in the hybrids producing fewer viable eggs than the parent strains.

Taylor and Smith (1956) studied malathion-resistance in susceptible and resistant substrains of the Cranbury-1 strain of T. urticae, which

originated from a greenhouse at Cranbury, New Jersey. Resistance was found to be due to a dominant gene transmitted by either sex. Reciprocal crosses between resistant females and susceptible males resulted in all F_1 females being resistant. The F_1 males from the resistant female parents were all resistant, indicating that the female parents had been homozygous for the dominant resistance factor, for had they been heterozygotes then half of the F_1 males and half of the females would have been susceptible. The F_1 males obtained from the susceptible female parents were all susceptible. The authors found no evidence of maternal effects. A 1:1 ratio of susceptible and resistant types were found in the offspring of the backcross (R ♀ x S ♂) x S♂ when they were treated by a discriminating dosage of malathion, and from this result it was concluded that malathion-resistance in T. urticae was due to a single dominant gene.

The inheritance of parathion-resistance was studied by Andres and Prout (1960) in another tetranychid, T. pacificus McG., obtained from cotton and alfalfa in the San Joaquin Valley, California. Similar reciprocal crosses between parathion-resistant and susceptible strains, and the 1:1 ratio thus obtained from the backcross of the F_1s with susceptible mites, led to the conclusion that parathion-resistance in this species is also under the control of a single dominant gene transmitted by either sex. In this case, however, a lack of linearity in the F_1 dosage-mortality regression line was taken to indicate the existence of a modifier factor or factors in addition to the major dominant gene. In another experiment Andres and Prout selected their S strain with one high dose of parathion to obtain about 99% mortality. The resulting

population, consisting of the progeny of the survivors, was found to have approximately 54% susceptible and 46% resistant individuals. Since such a progeny ratio would be produced by a surviving R+ female heterozygote, already fertilized by a susceptible + male, this result was therefore considered as another indication of a simple gene combination, or more likely, a major gene for resistance.

Dittrich (1961, 1963 a,b) developed a highly resistant R substrain of the Leverkusen strain of T. urticae by means of 30 selections with oxydemetonmethyl (methyl demeton). He concluded that resistance in this strain was caused by numerous dominant semi-lethal genes inviable when homozygous. He reported that inbreeding removed the dominant resistance factors, leaving a recessive resistance gene (termed r) which could also be made homozygous by further inbreeding and low selection pressure to remove the heterozygotes. He further concluded that the dominant semi-lethal genes as well as the homozygous recessive genes caused a loss in vitality of the resistant strain; this was manifested in a decreased fecundity especially at higher temperatures, a longer development period, and reduced voracity, a weaker resistance to starvation, a lower tolerance of high temperatures and a sex ratio distorted to favour males.

The results of Taylor and Smith (1956) were confirmed by the work of Helle (1962), who studied the inheritance of parathion-resistance in two resistant Leverkusen strains, one selected with demeton, and concluded that it was due to a major dominant gene transmitted by either sex. He found that reciprocal crosses of the parent resistant strain with a susceptible strain gave female progeny that were all resistant.

When the F_1 females from either cross were backcrossed with susceptible males, the females among the backcross offspring were found to be of two types in a 1:1 ratio, thus indicating a dominant gene transmitted by both sexes. The major dominant gene was isolated from possible modifier genes by repeated backcrossing combined with low-pressure selection with parathion; the repeated backcrossing of the F_1 hybrids with susceptible males substitutes alleles from the susceptible strain for minor resistance factors, while the low selection pressure removes the minor resistance factors along with the susceptible allele of the main resistance gene. The resulting strain, Leverkusen SP, so obtained was found to be 4 times less resistant to diazinon than the parent demeton-resistant strain. It was therefore concluded that some of the resistance in the parent strain had probably been due to minor modifier genes. Helle (1965a) discussed in detail the discrepancies between his results and those of Dittrich on strains from the same Leverkusen colony. Dittrich's (1963b) experiments were carried out with a Leverkusen strain, he selected with oxydemetonmethyl (M-Systox-R) and having a slope value of about 1. Such a low slope indicated that this strain contained an appreciable proportion of susceptible mites and it is entirely possible that had it been tested at a wider range of dosages a compound response curve would have resulted. No points were shown on the ld-p line for this strain, there was no indication of the statistical accuracy of the line, and the line did not ascend beyond the 70% mortality point.

A resistant inbred population (I) was obtained by Dittrich by mating individual females from the parent R strain with their brothers.

After 6 generations of such sib-mating, one female and one male from each of 5 inbred substrains were pooled and allowed to interbreed for 3 generations. A test of the resulting "population" with oxydemeton-methyl showed it to be heterogeneous for resistance; the compound response curve that resulted had a plateau at about the 50% mortality level indicating a 1:1 ratio of susceptible and resistant individuals. Dittrich (1963b) expected a straight, steep ld-p line from this strain. He did not analyze this population genetically as is normally done by crossing females from it with susceptible males and testing the F_1 progeny, to determine whether the resistance was dominant or recessive, but instead he selected this inbred strain with 0.005% oxydemetonmethyl. "After the population had been permitted to build up" (3 generations after selection) it was tested with the same concentrations of the toxicant as were used to test the original inbred resistant strain I, and a straight, steep ld-p line resulted, indicating that the susceptible mites (which Dittrich termed "low-resistant") had been selected out of the I strain. Dittrich assumed that the selected-strain had become homozygous for a recessive gene, but it could just as well been assumed that it contained few or no susceptible homozygotes but mostly individuals heterozygous and homozygous for a dominant resistance gene. His assumption was based on the fact that if the resistance allele was recessive, then the susceptible allele (+) would be selected out faster since the susceptible allele would be removed by the mortality of the +r genotype as well as the ++ genotype by the discriminating dose. Helle (1965a) calculated that if the resistance gene was recessive the susceptible allele would, indeed, be eliminated by the third generation

after selection but if resistance was dominant only 7% of this allele would remain in the selected population; therefore, there is no reason for Dittrich's statement that the "low-resistant class" in his inbred and pooled strain I, "had to be constituted of +r and ++ animals". In another experiment Dittrich (1963b) pooled "4 surviving inbred lines" which he termed "fast developing"and 4 similar inbred lines termed "slow developing" to produce two other inbred strains IIa and IIb respectively. When tested with oxydemetonmethyl, strain IIb gave a 7:3 ratio of susceptible to resistant mites, whereas the IIa strain gave a straight ld-p line similar to that obtained for the original inbred resistant strain after selection with 0.005% oxydemetonmethyl. He speculated that the differences between these two "populations" might have been due to environmental factors. Dittrich assumed that "the four fast developing lines represented" in strain IIa "obviously" were homozygous for the recessive gene but "the four slower lines" in strain IIb "still contained heterozygotes for the recessive r gene or normal individuals". From the results presented by Dittrich it is equally likely that strain IIa contained mites of the genotypes RR and R+ for a dominant resistant gene and that strain IIb contained about 70% of mites homozygous for susceptibility ++. Dittrich's conclusions appear to be based on speculation rather than on genetic crosses of resistant and susceptible mites and the determination of the genotype of the F_1 hybrids which result. The 1:1 ratio he obtained on two occasions with tests of his inbred strains from pooled, sib-mated females from the parent R strain is readily explained by the fact that a portion of the individual females chosen for inbreeding must have

after selection but if resistance was dominant only 7% of this allele would remain in the selected population; therefore, there is no reason for Dittrich's statement that the "low-resistant class" in his inbred and pooled strain I, "had to be constituted of +r and ++ animals". In another experiment Dittrich (1963b) pooled "4 surviving inbred lines" which he termed "fast developing"and 4 similar inbred lines termed "slow developing" to produce two other inbred strains IIa and IIb respectively. When tested with oxydemetonmethyl, strain IIb gave a 7:3 ratio of susceptible to resistant mites, whereas the IIa strain gave a straight ld-p line similar to that obtained for the original inbred resistant strain after selection with 0.005% oxydemetonmethyl. He speculated that the differences between these two "populations" might have been due to environmental factors. Dittrich assumed that "the four fast developing lines represented" in strain IIa "obviously" were homozygous for the recessive gene but "the four slower lines" in strain IIb "still contained heterozygotes for the recessive r gene or normal individuals". From the results presented by Dittrich it is equally likely that strain IIa contained mites of the genotypes RR and R+ for a dominant resistant gene and that strain IIb contained about 70% of mites homozygous for susceptibility ++. Dittrich's conclusions appear to be based on speculation rather than on genetic crosses of resistant and susceptible mites and the determination of the genotype of the F_1 hybrids which result. The 1:1 ratio he obtained on two occasions with tests of his inbred strains from pooled, sib-mated females from the parent R strain is readily explained by the fact that a portion of the individual females chosen for inbreeding must have

been susceptible for a dominant gene. The great heterogeneity (very low slope) of his parent R strain indicates that this could easily have been the case.

Helle (1965a) also questioned the conclusion of Dittrich (1963a) that the genes for resistance involved a depressed vitality. While conceding that vitality was indeed depressed in the Leverkusen RR strain, he was of the opinion that it derived from a genetic instability induced by the extremely high selection pressure Dittrich applied. That the strain was extremely heterogeneous was indicated by the shallow slope of its dosage-mortality regression line and by the relatively rapid loss of resistance when it was removed from selection pressure. A homogeneous strain of homozygotes for resistance such as the strain SP, obtained by Helle, showed no deficiencies in vitality. In a personal communication Helle (1966) has stated that the fitness of the OP resistance allele of the major gene is almost as great as that of its normal wild-type allele, and is in no way deleterious.

Schulten (1966) studied the inheritance of resistance to parathion and oxydemetonmethyl (demeton-S-methyl) in a multiresistant Baadse strain of T. urticae from a nursery in Holland, and in the Leverkusen SP strain of Helle (1962). A susceptible Sambucus strain of T. urticae was obtained from the Voorne area of Holland. By a series of backcrosses of this strain with the resistant Baadse strain followed by mild demeton selection, he found that the level of resistance to oxydemetonmethyl was reduced but the parathion-resistance remained in 50% of the mites. To discover whether both resistances were controlled by this dominant gene, which he called OP, backcrossing and selection

was continued but with mild parathion selection. This writer concluded that the OP-resistance was due to a dominant factor controlling a "large" resistance to parathion and a "smaller" resistance to oxydemetonmethyl. He also concluded that modifiers for the major OP factor increased the level of resistance to oxydemetonmethyl.

A relationship has been found between OP-resistance and diapause in T. urticae by Saba (1961b), who studied the production of diapausing forms in four strains with different degrees of resistance. Under constant conditions of 9 hours light per diem, a temperature of 13°C and an R.H. of 85%, the percentage of females entering diapause was much lower in the OP-resistant strains than in the susceptible strain, and the negative correlation with the degree of resistance was quite consistent and progressive until diapause became completely absent in the most highly resistant strain. Helle (1962) also found a subnormal incidence of diapause in his demeton-selected Leverkusen R strain, although it did not appear in his homozygous resistant SP strain. Therefore, he concluded that the reduced diapause in OP-resistant strains cannot be due to a pleiotropism of the major resistance gene, but it could derive from a linkage between the factors for nondiapause with the modifier genes for resistance. The nondiapause character may be selected in normal populations in a greenhouse by application of a reduced photoperiod and lower temperature without inducing any increase in OP-resistance. Thus the reduced diapause in OP-resistance strains may be due to the coexistence of two separate adaptations (Helle, 1965b).

Cross-resistance may be the pleiotropic expression of a single factor at the genetic level, so that resistance to one insecticide is

always linked with resistance to one or more insecticides in a given insect. In cases where cross-resistance extends not only within a group of compounds of similar chemical nature such as the OP insecticides but also to compounds of a different chemical type, a single enzymic detoxication mechanism may not be involved and therefore the resistance mechanism or mechanisms may be largely non-specific. Alternatively, two or more mechanisms may co-exist in the same insect (Winteringham, 1962). Cross-resistance from malathion to parathion and other OP compounds, on the other hand may be caused by the same enzyme; Matsumura and Voss (1964) found in T. urticae that it was due either to the same malathion carboxyesterase that caused the malathion resistance or to another OP-degrading closely related enzyme also produced by the genetic modification of the nonspecific aliesterase. Smissaert (1964) felt that the characteristically greater insensitivity to inhibition of the ChE of the OP-resistant Leverkusen strains was due to allelism in a single gene. Later Smissaert (1965) speculates that inversions disturbing normal crossing-over would result in the α-naphthylacetate hydrolysis factors (OP-degrading esterases) found in his experiments becoming completely linked with the cholinesterase factor, or that there is a single mutant gene with sufficient pleiotropism to produce both the OP-insensitive ChE and also the esterase enzyme or enzymes.

Recently the phenomenon of enzyme induction has been studied, whereby the storage and/or the toxicity of a poison may be grossly influenced by pre-exposure to another poison or drug. For example it has been shown that microsomes from the endoplasmic reticulum of animals pretreated with DDT or dieldrin actively hydrolyse paraoxon

(Triolo and Coon, 1966). Regulators of protein synthesis are implicated in the mechanism underlying this enzyme induction phenomenon (Chefurka, 1967). A theory proposed by Jacob and Monod (1961) from work on Escherichia coli suggests that several genes comprise an operon and these genes have related functions and are regulated together, so that a single molecule of messenger RNA transcribes the entire operon. The activity of the operon genes are regulated by a small region of the operon called the operator. Repressor substances, unknown, can inactivate the operator and prevent the formation by the operon of messenger RNA to synthesize enzymes. It is possible that certain effectors including some pesticides may inactivate the repressor substances and induce the operator to produce messenger RNA and thus enzyme formation. This phenomenon of enzyme induction of pesticide-converting enzymes by unrelated pesticides may well be the answer to these cross-resistances that cannot be otherwise explained, in that certain OP compounds and some unrelated compounds may inactivate the operator to varying degrees and thus induce enzyme production to a greater or lesser extent than others and account for the differences in the degree of cross-resistance (Chefurka, 1967).

To obtain a better understanding of the development of resistance in mites, Helle (1965c) looked for possible deleterious recessives in the susceptible Sambucus strain of T. urticae in Holland by inbreeding them repeatedly by means of sib-mating. The subsequent increase in homozygosity caused no general loss of vigor in any of the inbred lines, but apparently it resulted in a slight but significant increase in the number of non-viable eggs. The viability was less in the haploid eggs

from unfertilized females than in both haploid and diploid eggs from the fertilized females. The author did not discover any reason for these results, but suggested that possible explanations might include "the existence of a persistent heterotic system" in the mites, and "perhaps homozygosity of matroclinous factors having more effect on weaker haploid eggs".

Three visible marker genes, including a factor for albinism, have been found in a strain of T. pacificus. Existence of a linkage between this factor for pigmentation (p) and the single dominant factor (Rp) for parathion-resistance would aid in studies of the genetics of resistance. But no linkage was found between these genes since the factors Rp and rp in the resistant and susceptible types segregated independently of the factors p^+ and p in the pigmented and albino types (van Zon and Helle, 1966).

The inheritance of OP-resistance in insects appears to be similar to that in mites; a high level of resistance usually depends on allelism in a single major dominant gene, but several modifier genes may contribute to the level of resistance during the initial stages of development of the character. In houseflies OP-resistance is apparently inherited by a single normal gene which has a series of multiple alleles, one controlling malathion-resistance and another producing resistance to parathion and diazinon. The alleles convert normal aliesterase enzyme into modified and rather specific OP-degrading enzymes of the phosphatase or carboxyesterase type (Oppenoorth and van Asperen, 1960). The OP-resistance to paraoxon and diazoxon was found by these authors to be due to a modified aliesterase termed an

from unfertilized females than in both haploid and diploid eggs from the fertilized females. The author did not discover any reason for these results, but suggested that possible explanations might include "the existence of a persistent heterotic system" in the mites, and "perhaps homozygosity of matroclinous factors having more effect on weaker haploid eggs".

Three visible marker genes, including a factor for albinism, have been found in a strain of T. pacificus. Existence of a linkage between this factor for pigmentation (p) and the single dominant factor (Rp) for parathion-resistance would aid in studies of the genetics of resistance. But no linkage was found between these genes since the factors Rp and rp in the resistant and susceptible types segregated independently of the factors p^+ and p in the pigmented and albino types (van Zon and Helle, 1966).

The inheritance of OP-resistance in insects appears to be similar to that in mites; a high level of resistance usually depends on allelism in a single major dominant gene, but several modifier genes may contribute to the level of resistance during the initial stages of development of the character. In houseflies OP-resistance is apparently inherited by a single normal gene which has a series of multiple alleles, one controlling malathion-resistance and another producing resistance to parathion and diazinon. The alleles convert normal aliesterase enzyme into modified and rather specific OP-degrading enzymes of the phosphatase or carboxyesterase type (Oppenoorth and van Asperen, 1960). The OP-resistance to paraoxon and diazoxon was found by these authors to be due to a modified aliesterase termed an

"oxonase", produced by a mutant gene associated with a low level of aliesterase activity. Matsumura and Hogendijk (1964), on the other hand, found an enzyme "thionase" in resistant houseflies of the Ka and C strains which rapidly hydrolysed parathion and diazinon to diethylphosphorothionate, the principal degradation product in both susceptible and resistant flies, although this enzyme showed only low activity against paraoxon in the resistant strains. These authors could only speculate that both mechanisms of resistance may occur in houseflies and if so monogenetic control would be possible if both mechanisms were controlled by the same chromosomal locus. A group of 'modified' enzymes in the resistant strains may be the result of a single gene mutation but one or more minor factors may also be involved. Allelic genes may differ in the degree of resistance they produce in different OP compounds. Inheritance of a specific malathion resistance in Culex tarsalis was found to be due to a single semi-dominant gene allele which apparently increases the production of a carboxyesterase enzyme which is more heat labile and otherwise slightly different from the normal (Matsumura and Brown, 1963).

In the housefly, Franco and Oppenoorth (1962) found that the mutant a allele for diazinon-resistance was located on chromosome 5 very close to marker genes ar (aristapedia) and cm (carmine eye) and was completely linked with the ar gene. The mutant a gene conferring resistance to parathion in one strain of housefly and to malathion in another strain were both found to be closely linked with the R-DDT gene for kill resistance on chromosome 5. The two OP-resistances were found to show 4% crossing-over with the R-DDT gene which is very

close to the marker genes ar and cm (Hoyer, Flapp & Orchard, 1965).

The various factors that affect the susceptibility of normal strains of insects to test dosages of insecticides have been reviewed by Busvine (1957), Hamon and Mouchet (1961) and Pal and Kalra (1966), and include age, temperature, relative humidity and nutrition. Both pre- and post-treatment temperature have long been known to influence the toxicity of poisons. Das and Needham (1961) found that a change in post-treatment temperature can affect their course of action, which usually increases with increased temperature, or their ultimate toxicity.

Very few studies have been made of the effects of various factors on toxicity of compounds to mites. Harrison and Smith (1961) determined that the toxicity of dicofol to eggs of T. urticae increased as the relative humidity was decreased from about 60% to dry conditions. Between 60% to 95% R.H. the mortality increased slightly, whereas from 95% to 100% R.H. a sharp increase in mortality ensued. Intersexual differences were studied by Saba (1962), who found that males were 20 times more susceptible to TEPP than females in T. urticae. There are differences between the development stages of T. urticae in their susceptibility to acaricides; newly-moulted larvae are very susceptible to many OP compounds whereas the quiescent forms are very tolerant (Armstrong et al. 1954, Mailoux and Morrison 1962).

Fisher and Hansell (1964) studied the effect of pre-treatment and post-treatment temperatures on the toxicity of dicofol to T. urticae, and found that the mortality increased by 1.2% for each 1°F. rise in post-treatment temperatures from 50°F. to 90°F. Differences in

pre-treatment temperatures had no significant effect on the mortality.

Kensler (1965) studied factors affecting the vapour toxicity of DDVP to T. urticae. The age of the mites was the most decisive factor, older mites being least susceptible but host plant, temperature and relative humidity also had some effect on the susceptibility to the fumigant. Mites reared on tomatoes were 30% more susceptible than those reared on bean or cotton. The susceptibility level was similar when the rearing temperature and relative humidity were low (70°F, 35%) to the level obtained when they were high (90°F, and 88%). They were also negligibly different when the post-treatment conditions were either low or high with respect to temperatures and relative humidity. Parallel results have been reported in the susceptibility of houseflies to diazinon, which decreases with age in both diazinon-resistant and susceptible individuals; this was found to be associated with a slower cuticular penetration of the compound in the older flies (Farnham et al. 1965).

Henneberry (1960) studied the effect of nutrition on the fecundity of susceptible and OP-resistant strains of T. urticae and on their susceptibility to malathion; fecundity was lower in both strains when they were reared on host plants growing on low levels of nitrogen. The mortality on exposure to malathion was highest for mites fed on plants high in nitrogen when these were of the resistant strain, but the reverse was observed with the susceptible strain. Saba (1961a) found that resistance to TEPP developed at significantly different rates in strains of T. urticae reared on different hosts. He did not look for correlation between the mortality and the fecundity with the

nutritional level, but a relationship is likely since both Henneberry (1960) and also Grebel'sku and Bychenkova (1962) found that houseflies fed on milk were more tolerant of chlorinated insecticides than those fed on sugar.

Mites of the species T. urticae have been found to resemble insects and mammals in maintaining a daily rhythm in their response to various chemicals (Cole and Adkisson, 1964). Mites of the Blauvelt resistant strain of T. urticae reared in alternating light and dark conditions showed a maximum susceptibility to DDVP 2 hours after dawn, and a minimum susceptibility 2 hours after dusk (Polcik et al. 1964). Similar results were reported by Kensler (1965) for a susceptible strain of T. urticae reared under 12:12 or 24:0 hour light-dark cycles, with fluctuating temperature and relative humidity, and then treated with DDVP vapour. However, these rhythms could not be reproduced consistently, and he obtained just the opposite result with strains reared under similar light-dark cycles but with constant temperature, and constant or fluctuating relative humidity. Kensler could not account for this 12-hour shift in these rhythms. Fisher (1967) detected a circadian rhythm in the susceptibility of the Niagara susceptible strain to dicofol.

MATERIAL AND METHODS

1) Insecticide Chemicals

The following OP compounds were supplied as unformulated technical grades of insecticide by the following companies:

parathion, O,O-diethyl O-p-nitrophenyl phosphorothioate, Cyanamid of Canada Ltd., Rexdale, Ont.

demeton, mixture of O,O-diethyl S-(and O)-2-(ethylthio) ethyl phosphorothioates, Chemagro Corpn., Albany, N. Y.

fenthion, O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate, Chemagro Corpn.

malathion, diethyl mercaptosuccinate S-(O,O-dimethyl phosphorodithioate), Cyanamid of Canada Ltd.

ethion, (O,O,O',O'-tetraethyl S,S'-methylene bisphorodithioate, Niagara Brand Chemicals, Burlington, Ont.

dimethoate, O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate, Cyanamid of Canada Ltd.

mevinphos, methyl 3-hydroxycrotonate dimethyl phosphate, Shell Canada Ltd., Toronto, Ont.

Bidrin®, dimethyl phosphate, ester with cis-3-hydroxy-N,N-dimethylcrotonamide, Shell Canada Ltd.

phosphamidon, dimethyl phosphate, ester with 2-chloro-N,N-diethyl-3-hydroxycrotonamide, Chevron Chemical (Canada) Ltd., Oakville, Ont.

malaoxon, diethyl mercaptosuccinate, S-ester with O,O-dimethyl phosphorothiolate, Cyanamid of Canada Ltd.

The chlorinated acaricide dicofol, 4,4'-dichloro-a-(trichloromethyl) benzhydrol, was supplied as a technical-grade material by Rohm & Haas Co. of Canada Ltd., West Hill, Ont. The carbamate Temik®, 2-methyl-2-(methylthio) propionaldehyde O-methylcarbamoyl) oxime, was supplied by the Union Carbide Corp'n., New York in the form of 10% granules, the purified compound being too toxic to handle (acute oral LD_{50} for rats 0.9 mg/Kg.).

Radioactive C^{14}-malathion was obtained from the Radiochemical Centre, Amersham, Bucks, U. K. through the generosity of the World Health Organization.

2) Biological Strains

Five strains of Tetranychus urticae were employed during this investigation; 4 of them were derived from the parent Niagara susceptible colony (NS). This strain was obtained from Niagara Brand Chemicals, Middleport, N. Y. in 1963, and originated from a collection made at Gasport, N. Y. in the early 1950's; it had not been previously exposed to any OP compound or specific acaracide.

The Niagara parathion-resistant strain (NR) was selected at Middleport from the NS strain with parathion, plus a little selection with demeton in the early generations, and was reported to have stabilized at a 40-fold resistance to parathion. The other 3 strains were selected from these strains by the author. During most of this investigation, selection pressure was maintained on the NR strain at the 85 - 95% mortality level by treating it with

0.15% parathion at intervals of approximately 3 weeks, but only a portion of the culture was treated each time to guard against loss of the colony.

The 'intermediate' resistant strain (I) was selected from the NS strain by selecting it with 0.045% parathion (i.e. at the 80% mortality level) every 3 weeks for a 3-month period. These strains were used in studies on the inheritance of resistance.

For the cross-resistance studies a third resistant strain (NBR) was selected from the Niagara NS strain with 0.0014% Bidrin for 10 generations; the selection pressure exerted mortalities between 95% and 99% in each generation.

A fourth strain (NRBR) was obtained by selecting a sample of the parathion-resistant NR strain with Bidrin for the same period, with the same final concentration. The method of obtaining a fifth strain, namely the susceptible NS_{21} line, will be described under section 2 i.

3) Rearing Methods

Extreme precautions are necessary to avoid contamination of individual cultures (Hansen, 1958). In this study the cultures were reared under constant fluorescent light on scarlet runner beans cut back to two primary unifoliate leaves. Each culture was isolated within wire-screen cages which were coated with a sticky polybutene compound and placed over water-filled pans (Fig. 1). The stems of the host plants were also ringed with a sticky adhesive material. During the maintenance procedures, susceptible strains were always handled before the resistant strains, to avoid the

Fig. 1. Wire-screen cages coated with polybutene, and the water-filled pans used to isolate the different strains of T. urticae during rearing on Scarlet-runner bean plants.

reverse contamination. The host plants had been grown over water-filled isolation pans and carefully examined for mites before being used for cultures. As a further precaution, the susceptible strains were kept in a separate building from the resistant cultures. Fresh host plants were added to each culture as required, usually twice a week. Later generations of the susceptible strain were reared in separate controlled-environment cabinets at 24°C and 60% R.H. (Fig. 2).

Except where otherwise mentioned, only adult females were used in the experiments with the various strains. To ensure an adequate supply of adult female mites of approximately the same age for each test, several hundred ovipositing adults were put on uninfested bean plants for 48 hours. Their progeny matured in about 13 days at a temperature of 24°C - 29°C and were used when about 3 - 5 days old. For experiments where close observation of small numbers of mites was required, i.e. fecundity and egg-viability experiments, the mites were reared on discs of bean leaf (Fig. 3); these were placed on damp cellucotton, soaked with tap water being found to be just as effective as the cellucotton soaked with nutrient solution employed by Helle (1962).

4) Toxicological Test Method

The OP compounds employed to test the mites in the genetic experiments were dissolved in a 1:20 mixture of olive oil and acetone, and diluted with the mixture to obtain the series of concentrations evenly spaced on a logarithmic scale. Tests with the oil-acetone solvent alone were made as a control. For most cross-resistance tests it was found that acetone alone was satisfactory

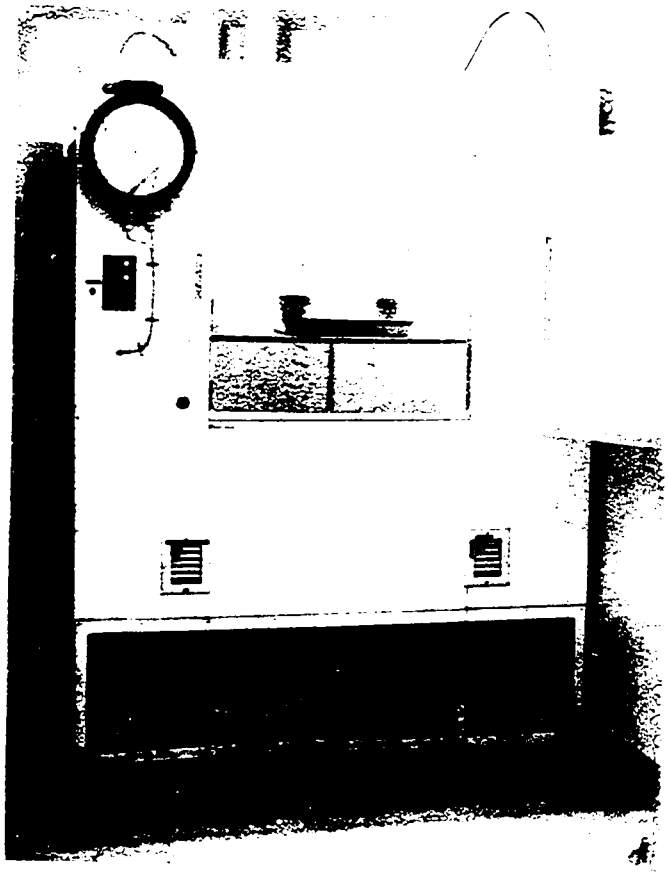


Fig. 2. Controlled temperature and humidity cabinet used to rear the susceptible NS and [illegible] strains during part of this investigation.

as the solvent for all compounds tested.

For exposure to the pesticide, adult female mites were placed upside down on Scotch Brand adhesive tape fastened to a microslide (Fig. 4), a method modified after Voss (1961). The slide was sprayed for 12-15 seconds in a Potter Spray Tower (Fig. 5) with a 5-ml aliquot of toxicant. Air pressure at the nozzle in the tower was maintained at 15 cm. of mercury. An exhaust fan removed excess spray mist, and the tower was further modified after Harris et al. (1962) by enclosing it in glass panels.

The sprayed mites on the glass slides were placed over distilled water in a sealed desiccator jar almost immersed in a water bath at a temperature of 23.9$\pm$ 0.01°C (Fig. 6), controlled by a Bronwill constant temperature unit with a mercury thermoregulator. The temperature within the desiccator did not fluctuate more than 0.2°C, as demonstrated by a portion of a potentiometer recording taken during a 24-hour test (Fig. 7). Humidity was measured by means of a small fan-driven psychrometer designed by the author (Herne, 1967). Mortality counts were made with a stereoscopic microscope 24 hours after treatment. Mites which failed to respond with sustained leg movements after being prodded with a sable-hair brush were considered to be dead.

Dittrich (1962), in comparing different test methods, found a lack of homogeneity between the sprayed replicates. Therefore, in order to obtain an estimate of the variability between sprayings in the method used in this investigation, the mites were tested with parathion at any one dosage in 5 successive sprayings of 10 mites at a time. There were thus 10 mites per slide and 50 mites per dosage

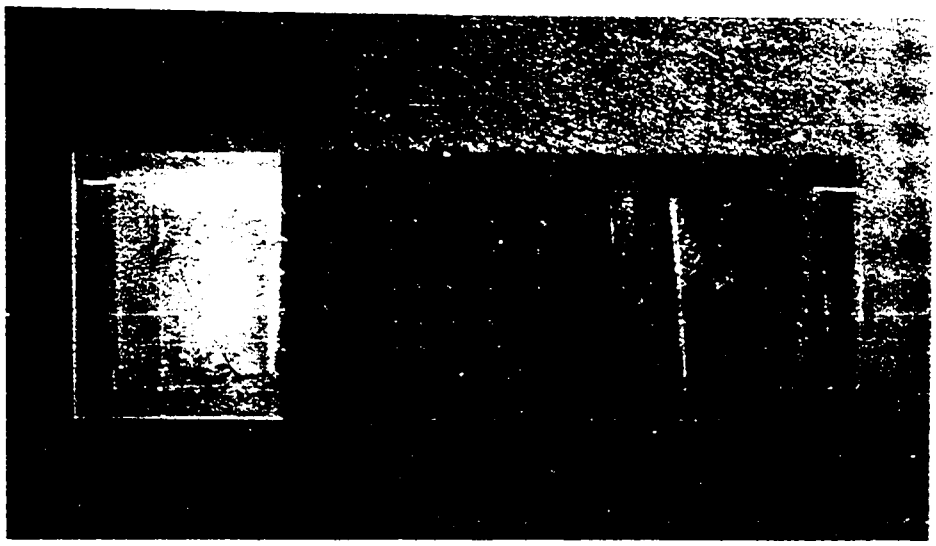


Fig. 4. Adult female mites of uniform age, upside down on taped-slides prior to treatment in the Potter Tower in dosage-mortality tests.

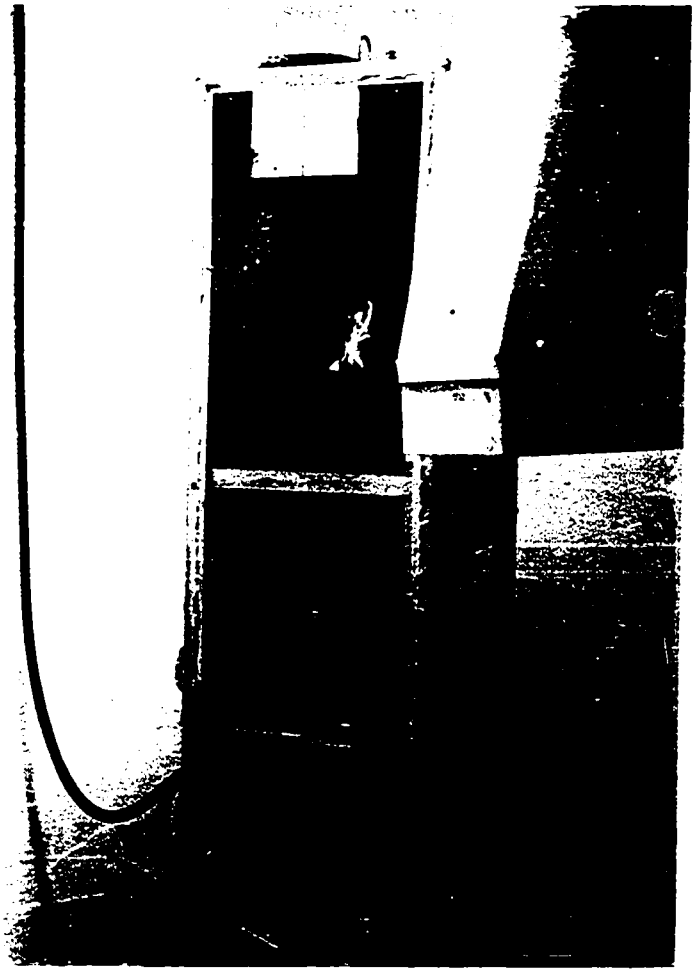


Fig. 5. Potter Spray Tower used to treat female mites on taped-slides with scalar doses of toxicant in dosage-mortality tests.

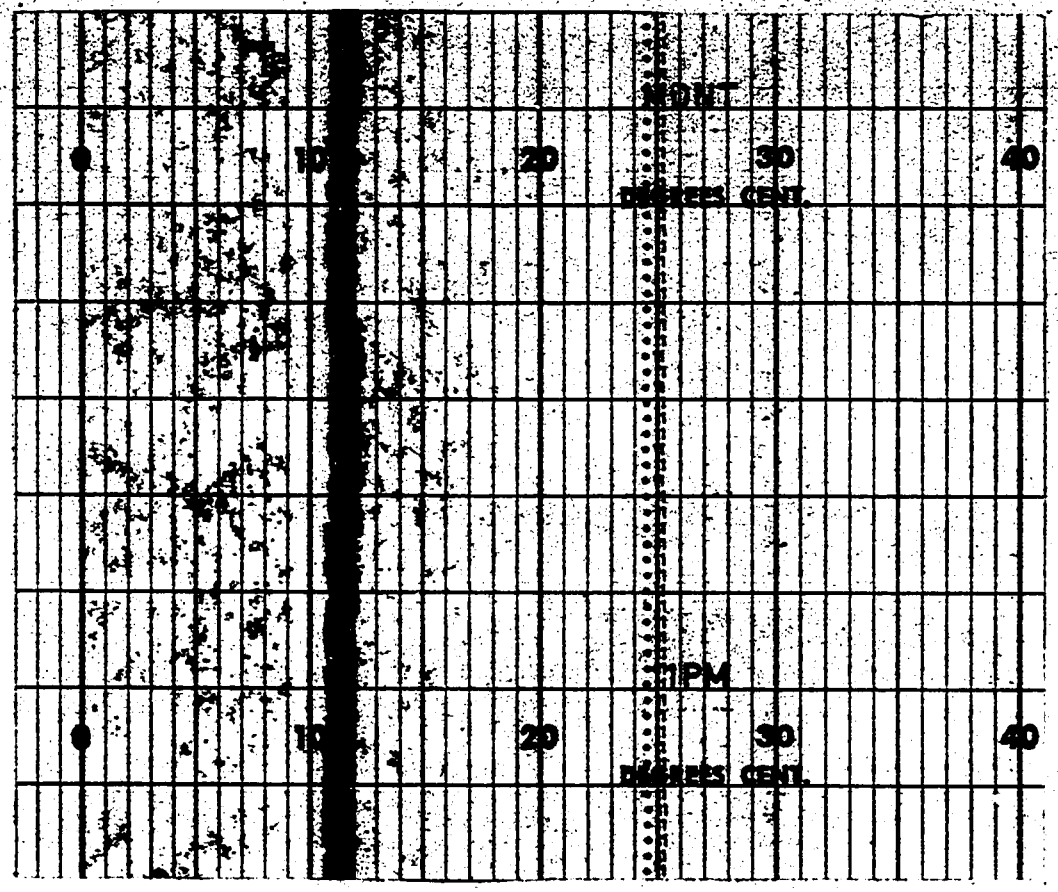


Fig. 7. A portion of a 24-hour recording of the temperature within a sealed desiccator held in a water bath controlled by a Brownell thermoregulator accurate to $\pm$ 0.01°C., indicating the accuracy of the temperature control.

in each test, and the tests were repeated on 3 additional days, making 4 tests in all.

Dosage-mortality data were corrected with Abbott's formula (Abbott, 1925) and analysed by a 1620 computer programmed for probit analysis at the Statistical Research Service, Canada Department of Agriculture, Ottawa. This was possible only for straight or nearly straight log-dosage probit-mortality regression lines (ld-p lines) or straight portions of compound response curves. Since the computer made no allowance for lines that fell off slightly in the high mortality range, the actual slope of such lines is probably slightly steeper than that indicated by the relation between their LD_{50} and LD_{95} figures. However, the computed LD_{50} figures showed very little difference whether or not allowance was made for the slight levelling off of some ld-p lines, and the difference was very much less than the variation between replicate experiments; moreover, it was insignificant as compared to the gross differences between the susceptible and the resistant strains. The LD_{50} and LD_{95} points determined by the computer were plotted on 3-cycle or 4-cycle log-probit paper, and the ld-p lines were drawn by joining them.

For the cross-resistance studies carried out on each strain, each compound was submitted to 2 to 6 replicated tests, thus involving 150 mites per concentration on the average. To determine resistance levels in the cross-resistance study, the LD_{50}'s of the susceptible strain and the resistant (test) strain were obtained from the computer analysis of the dosage-mortality values for a

given compound. The level of resistance for the various compounds was expressed as the ratio of the LD_{50}'s $\frac{LD_{50}\text{ resistant}}{LD_{50}\text{ susceptible}}$.

In assessing the cross-resistance to Temik of the susceptible NS and resistant NR strains, the 10% granular formulation was applied to the soil under mite-infested bean plants at rates of 0.115 and 0.23 gm per the 4-inch pot in which they were grown. Tests on each strain consisted of 2 replicates at each rate. The counts for mortality were made 48 and 72 hours after the Temik granules had been applied, by taking the first 300 mites to be found and categorizing them as alive or dead.

After the susceptible NS strain had been selected with Bidrin for 10 generations, the resulting NBR strain was tested for any change in its resistance level to Bidrin and for its cross-resistance to parathion and phosphamidon. The resistance levels of the Bidrin-selected strain were compared with those obtained with the parathion-selected NR strain to determine whether both selecting agents induce similar cross-resistance patterns. To determine whether the initial low level of cross-resistance to Bidrin in the parathion-selected NR strain could be increased, a sample of this strain was selected with Bidrin for 10 generations, as previously described, to produce the NRBR strain, and the LD_{50}'s for parathion and Bidrin were then determined.

5) Conditions of Test

The various factors influencing the mortalities obtained with the test method were assessed in order to determine the degree of standardization necessary for an accurate investigation of

OP-resistance. It was not possible however, to replicate all of these assessments, and precise temperature and humidity conditions were not always available.

The effect of pre-treatment temperature on mortality was determined by holding 50 adult females of the resistant NR strain for 24 hours at 26.7°, 23.9° and 7.2$\pm$ 5°C, each at 95 - 100% R.H., and then testing them with dosages of 2.74% and 5.38% parathion.

To test the effects of post-treatment temperature and relative humidity, groups of 50 adult females of the NS strain were treated with 0.050% parathion and held at temperatures ranging from 12°C to 28$\pm$ 5°C and relative humidities ranging from approximately 42% up to 100%.

To test the effects of post-treatment and humidity on mortality, groups of 50 NR females treated with 3.84% and 5.38% parathion were placed in sealed evaporating dishes 130 mm in diameter, held at relative humidities ranging from 32.8% up to 100% by means of the various saturated salt solutions of Winston and Bates (1964); the dishes were held for 24 hours in sealed desiccator jars immersed in a water bath at 23.9$\pm$ 0.02°C. A possible fumigant effect within the sealed containers was tested by placing a slide carrying NR mites treated with solvent only alongside a slide carrying NR mites treated with a high dose of 7.53% parathion in a small jar (405.6 cu. cm.) held for 24 hours at 24°C and 95 - 100%R.H.

To determine the effect of the age of the mites on the test mortality, groups of females of the susceptible NS strain differing in age from newly-moulted adults to 15-day-old adults were treated

with mevinphos (0.0018%) under normal conditions and examined for mortality 24 hours later. In another test, adults 2-4 days old were compared with adults 7-15 days old under identical test conditions. In addition adults respectively 7, 14 and 31 days old were tested simultaneously with a logarithmic series of parathion dosages ascending from 0.013 up to 0.1% concentration.

The effect of the time of day at which the treatment was applied was tested by spraying groups of 50 adults of the NS and NR strains with 0.035% and 2.74% parathion at 1-hour and/or 2-hour intervals within a single day, starting at 6:20 a.m. and finishing at 6:30 p.m.

During studies with malathion it was found that over a certain range an increase in the dosage resulted in a slight decrease in mortality, thus producing a dip in the plateau region of the dosage-response curve (Fig. 8). The same phenomenon was shown by 2 other OP compounds which are liquid, namely ethion and dimethoate (see Appendix 2). With malathion it was observed that mites treated with the higher dosages were visibly coated with the liquid insecticide; therefore, the possibility was investigated that the greater viscosity of the higher concentrations was giving some protection to the mites. Two different concentrations of malathion (0.1 and 1%) were made approximately equal in viscosity by adding 0.9% corn oil to the lower concentration. These solutions were compared to each other for mortality and with 0.1% malathion without oil, as well as with corn oil alone. A similar experiment was conducted with parathion using olive oil to increase the viscosity of the lower dosage of parathion.

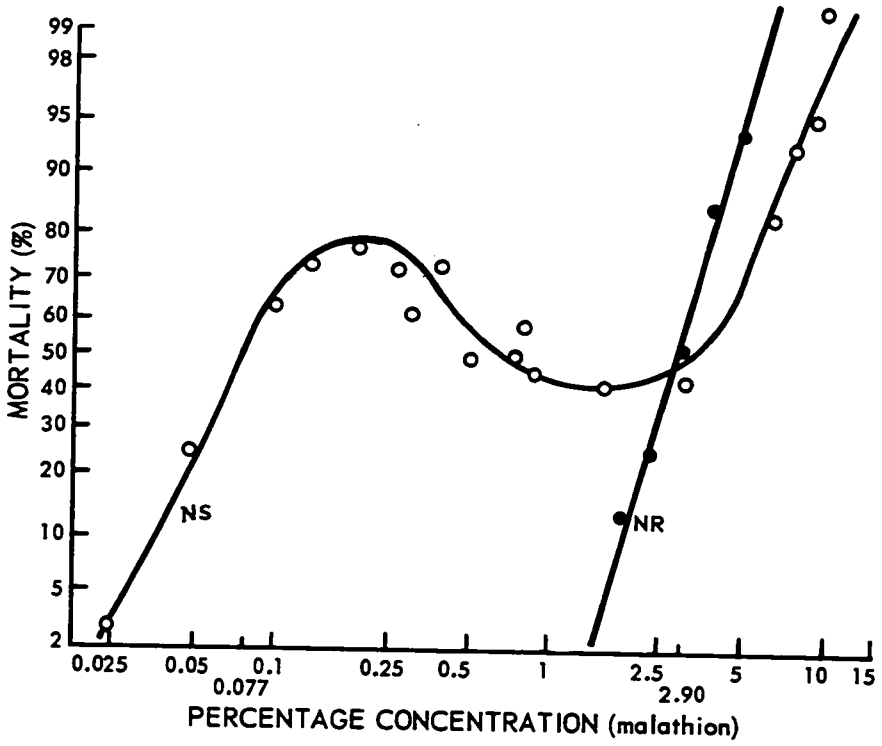


Fig. 8. Dosage-mortality relationships to malathion for the NS strain when tested with 18 closely-spaced dosages, compared to the line for the NR strain.

The speed at which mortality ensued was also studied by means of 0.2% and 1.6% malathion solutions applied to groups of 10 adult females. Their response was assessed over a period of 72 hours after treatment by prodding them at intervals and ascertaining whether they still responded by leg movements. In another test 2 groups of 25 females were similarly treated and observed at hourly intervals for a 24-hour period after treatment.

6) Tests for Genetic Purity

Several methods were developed to test the homogeneity of the susceptible NS strain. In the female discriminating-dose test an individual female was crossed with a susceptible male. The fertilized female was isolated on a leaf disc, and half of her female progeny were treated by the slide-spray method with a discriminating dose of 0.34% parathion. If these F_1 females proved susceptible, a strain was reared from their untreated sisters. By this method a second susceptible strain, termed the NS_{21}, was obtained, and this was compared with the original NS strain.

In the male discriminating-dose test, a virgin female was mated with her sons after it had been first determined that a portion of them had shown 100% mortality when treated with 0.008% parathion, a discriminating dose which kills less than 20% of the resistant males. The female was surely then homozygous for the susceptible allele of the main resistance gene, since all the sons being haploid could only be + if 100% were killed by the 0.008% dose, and the mother could only be ++ to have produced such sons. All the F_1 females could therefore only be ++, homozygous for

susceptibility. The F_1 progeny of females, which were found to be homozygous susceptible ++ by this method, were compared with the parent-strain females by the slide-spray method. The male discriminating-dose method can also be employed to obtain either a homozygous susceptible strain or a homozygous resistant strain from a mixed population of resistant and susceptible mites.

Helle (1962) claimed that no suitable method exists for experimenting with male spider mites. But they can be tested by the slide-spray method if great care is taken in transferring them to the taped slides. The only handicap is that natural mortality is higher with male than with female mites.

To test further the purity of the NS strain, the few mites which survived high dosages of parathion (0.1%) were isolated on bean leaf discs and any female progeny obtained were compared on the same slide with females from the NS strain and from the NR strain at any one dose of parathion. In one test, 331 females of the NS strain were sprayed with parathion (0.002 to 0.0032%) while they were on pieces of bean leaf in dry petri dishes. The dishes were then covered with a fine nylon material to isolate the mites; 24 hours later each motile survivor was transferred to a clean bean leaf placed on wet cotton, and a mixture of their F_1 and F_2 progeny were treated with 0.1% parathion. It was possible to test a mixture of the F_2 and F_3 progeny of one female survivor removed directly from a taped slide which had been treated with parathion (0.1%). A dose of 0.34% parathion was used to compare these progeny with the original susceptible strain. Females could not

usually be removed from taped slides without injury and most of them died without ovipositing.

7) Genetic Crossing Experiments

Reciprocal crosses were made to determine whether resistance to parathion was inherited as a dominant factor. Niagara susceptible NS strain mites, referred to as S, were crossed with the parathion-resistant NR strain, referred to as R; females of the intermediate strain I were crossed either with R or with S males. By means of 2 or 3 screening tests, at least 5 serial dosages had been established within the 20 to 85% mortality range for the S and R strains respectively. The dosages employed for the F_1 offspring of the reciprocal crosses and the I♀ x R ♂ cross were similar to those employed for the R strain. For the I strain, composed of both susceptible and resistant individuals, and for the female offspring of backcrosses, additional concentrations were intercalated between these two ranges, to make a total of 14-19 dosages.

By means of a very fine sable-hair brush, at least 150 quiescent deutonymphs, ready to moult into adults, were transferred to 2 trimmed leaves of a clean bean plant. The petioles and stem of the plant were ringed with a sticky material. A slightly greater number of mature adult males was placed on the same leaves. Five days later the fertilized females were transferred to about 6 or 7 leaves on a clean bean plant and left to oviposit for 3 days. The original adult females were then removed from the plant and in some cases were used to produce a second batch of eggs. The plant carrying the eggs laid between the 3rd and 6th days, was held at

23 to 24°C until the F_1 females which developed from them on it were 3 - 5 days old, when groups of the required number of females were transferred to taped slides for testing with parathion.

Backcrosses of the F_1 females with both S and R males were made to determine whether the inheritance of parathion-resistance in the Niagara strain was monofactorial or polyfactorial. The B_1 females from such backcrosses should be susceptible or resistant in a 1:1 ratio if a single gene is segregating. A test of F_1 males from an R♀ x S♂ cross would also indicate monofactoriality if all males proved resistant. The F_1 males from crosses or backcrosses were not treated with parathion on taped-slides for reasons already mentioned.

The procedure for the backcrosses was similar except that the F_1 female progeny were allowed to develop only to the quiescent deutonymph stage and were then isolated on a clean plant with S males. The B_1 female progeny were then treated with parathion.

8) Bionomic Characteristics

To compare the fecundity and egg-viability of the resistant and susceptible strains, 20 newly-matured and fertilized females from each strain, within 2 hours of the same age, were isolated individually on leaf discs 3.5 cm in diameter placed in wet cotton in petri dishes. The eggs laid by each female after 2, 3, 9 and 15 days, and the percentage of non-viable eggs were recorded.

The possibility of differences in the heat-hardiness of the susceptible NS and resistant NR strains was studied by comparing their upper lethal temperatures at low (below 45%) and very high

(near 100%) relative humidities. In the low-humidity test the mites were placed inside vials, 25 mm wide by 75 mm high, which were sealed at the prevailing room temperature and humidity of 23°C and 45% R.H. As the temperature of the vials was raised toward a lethal temperature the relative humidity would of course be decreased. In the high-humidity test, distilled water was added to each vial to maintain approximately 100% R.H. Mites were secured upside down on Scotch Brand adhesive tape fastened around a section of 8-mm-O.D. glass tubing. Circular rubber spacers on the ends of the tubing centered the mites within the vial. Accurate control of temperature ($\pm$0.01°C) was achieved by totally immersing the sealed vials in the water-bath previously described (Fig. 1). Groups of at least 100 susceptible and 100 resistant mites were exposed for 24 hours, in both the low-humidity and the high-humidity tests, to temperatures of 39°, 40°, 41°, 42°, 43° and 47°C.

9) Malathion Detoxication Assays

For the experiments on mechanism of resistance the methods of Matsumura and Brown (1961, 1963), Matsumura and Voss (1964) and Voss and Matsumura (1964, 1965) were followed wherever possible.

The *in vitro* enzymatic degradation of C^{14}-malathion was compared with homogenates of the susceptible NS and resistant NR strains. Sample mites, mostly mature adults, weighing 60 mg were homogenized in a glass Potter-Elvehjem homogenizer in 3 ml 0.1 M sodium phosphate buffer at pH 7.4. The homogenizer was partly immersed in crushed ice to protect the enzymes. The C^{14}-malathion

was labelled on the 2,3-succinyl carbon and had a specific activity of 2.87 mC/mM (equivalent to 8.69 μC/mg). The benzene in the sample was evaporated off under nitrogen, and a stock solution was prepared containing 1230 μg C^{14}-malathion in 0.31 ml absolute ethanol. To each 20 mg wet weight of mite homogenate in 1 ml was added 0.01 ml of this stock solution to give 40 μg C^{14}-malathion. For each strain two replicates were run simultaneously. The homogenate and malathion were incubated in a shaker water-bath at 28°C for 30 minutes, after which 25 ml 0.2% trichloracetic acid in sodium phosphate buffer at pH 2.0 was added with continuous shaking to stop the enzyme reactions. The method of assessing enzyme activity (Matsumura and Brown, 1963) is based on the principle that at pH 2.0 the water-soluble fraction containing the phosphatase products can be separated from the chloroform-soluble fraction. Subsequently the carboxyesterase products are in turn rendered water-soluble by adjusting the pH to 7.0, and thus can be separated from the chloroform-soluble products. Therefore, the procedure was to extract the incubated homogenate twice with 25-ml aliquots of chloroform. The first portions of the chloroform fraction from 125-ml separatory funnel were centrifuged at 3000 r.p.m. for 10 minutes to aid in removing all the aqueous phase and some mite debris. The separatory funnel was washed with 10 ml distilled water to make a total of 35 ml of the aqueous fraction (a) containing the radioactive phosphatase products. The chloroform phase was filtered back into the separatory funnel through a glass-wool plug which was washed with 5 ml of the chloroform. The

chloroform fraction was adjusted to pH 7.0 by adding 50 ml of the appropriate aqueous sodium phosphate buffer and shaking. The clear chloroform fraction which contained the radioactive malathion, malaoxon, diethyl malate and diethyl 2-mercaptosuccinate, was run off into a flask. The remaining aqueous fraction (b) contained the carboxyesterase products.

The radioactivity in the 3 fractions was measured by means of a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 314M. The scintillation fluid for the aqueous phases was prepared by mixing dioxane 1000 ml, PPO (2,5-diphenyl oxazole) 12 gm, POPOP [2,2-p-phenylene-bis (5-phenyloxazole)] 0.6 gm, scintillation grade naphthalene 60 gm, and ethylene glycol 200 ml; 10 ml of this fluid was added either to 0.5 ml of the aqueous phase (a), or to 2 ml of the aqueous phase (b), in 25-ml screw-top vials. Background activity was measured by assessing control vials containing 10 ml of either scintillation fluid alone. Each vial was placed in the shielded well between two gamma detectors within a freezer compartment and cooled for 2 minutes before being counted. The scintillation spectrometer was adjusted to the settings recommended by the company for counts of radioactive C^{14} organophosphates, i.e. the high voltage was set at 3, and the power dial at 100. Each sample vial was counted for 1 minute and the counts were repeated 4 times to obtain an average. The counts on the upper 6 and the lower 6 counters were added together to give the total count, and the background counts (14 c.p.m. on the average) were subtracted therefrom.

Since the yellow colour of the chloroform fraction could interfere with the count of radioactivity by a quenching effect, the chloroform fraction was cleaned by the method of Storherr et al. (1964). The fraction was evaporated to dryness in a flash evaporator at a water temperature of 55°C. and redissolved in 25 ml of ethyl acetate. This solution poured into a column containing a glass-wool plug and a mixture of 5 gm norite, 4 gm magnesium oxide and 8 gm celite. The column was eluted with 200 ml of a 3:1 mixture of 25% ethyl acetate in benzene. The solution from the column was again evaporated to dryness in the flash evaporator and redissolved in 4 ml of pure toluene. A 2-ml aliquot was mixed with 10 ml of toluene scintillation fluid (Packard Instrument Co.) for counting. The count was compared with that obtained with the uncleaned sample to determine whether quenching had occurred; in this event it proved that there was no quenching effect.

10) Cholinesterase Sensitivity Assays

The ChE inhibition experiments are based on the principle that the ChE in the mite homogenates normally hydrolyses acetylcholine (ACh) substrate. Thus if a known amount of ACh is incubated with a sample of homogenate, the amount remaining after a given time will indicate the activity of the ChE. This amount can be measured colorimetrically when it has been converted to a hydroxamic acid ferric ion complex by adding alkaline hydroxylamine hydrochloride and ferric chloride (Hestrin, 1949). Thus the estimates can be made of the percentages of ChE inhibited by graded concentrations

of an OP compound to the system, and the concentration that inhibits 50% of the ChE (I_{50}) can be determined by reference to a standard curve for ACh hydrolysis.

Malaoxon, 99.9% technical, was used as the inhibitor to compare the sensitivity of the ChE's of the parathion-resistant and susceptible Niagara strains. Malaoxon was chosen because the inhibition rate of the ChE's of the Leverkusen resistant and susceptible strains differed more with this compound than with paraoxon. The procedure of simultaneous inhibition, in which the substrate ACh and the enzyme are incubated for the same length of time, was employed because it determines the competitive effect between the malaoxon inhibitor and the ACh, and because Voss and Matsumura (1964) considered that simultaneous inhibition was likely to occur naturally owing to the ACh already present in the mite tissues.

Mite homogenates containing 10 mg wet weight of fresh mites in 1 ml of 0.067 M phosphate buffer at pH 7.4 were prepared as previously described. From a stock solution of 10^{-2}M malaoxon in 3% acetone solvent, 13 concentrations of malaoxon were prepared such that their final concentrations in the 0.3 ml digests ranged from 10^{-3} to 5×10^{-8}M. These were incubated in 15 x 150 mm test tubes, and consisted of the following components: 0.24 ml of mite homogenate; 0.03 ml of malaoxon inhibitor; and 0.03 ml of ACh iodide, 2×10^{-2}M; making a final concentration of ACh substrate of 2×10^{-3}M. For a standard reading equivalent to 100% inhibition of the ChE, the homogenate and the malaoxon was replaced by 0.27 ml buffer solution, and for readings equivalent to 0% inhibition

0.03 ml buffer replaced the malaoxon. The ACh, and malaoxon were added to the homogenate by means of a 50-µl Hamilton syringe, and the digest was immediately incubated for 30 minutes at 30°C.

For the colorimetric assay of ACh the following reagents were used: hydroxylamine hydrochloride, 2M; hydrochloric acid, sp.gr. 1.18 diluted with 2 parts by volume of distilled water; sodium hydroxide, 3.5M; ferric chloride ($FeCl_3 \cdot 6H_2O$) 0.37M, in 0.1N hydrochloric acid. The method of Robbins et al. (1958) was followed except that the first three reagents were used at half volume as described by Bigley and Plapp (1960).

After the 30-minute incubation period, 2 ml of fresh hydroxylamine-sodium hydroxide (1:1) mixture was added to each test tube and shaken vigorously. Two minutes later 1 ml of the hydrochloric acid solution was added to each sample, followed by 1 ml of the ferric chloride solution, with vigorous shaking. The solutions were filtered through Whatman No. 40 filter paper into clean test tubes, and then transferred to 4-ml cuvettes for colorimetric estimation. The density of the yellow colour formed was determined at 540 millimicrons in a Beckman DU spectrophotometer, using a light path of 1 cm. A control for non-specific colour was obtained in each of the experiments by adding the hydrochloric acid solution before the hydroxylamine-sodium hydroxide mixture.

To test for a possible effect of malaoxon concentration and/or acetone on the optical density readings, 5 concentrations of malaoxon were incubated with phosphate buffer at pH 7.4 replacing

the mite homogenate. Neither malaoxon concentration nor acetone solvent were found to have any effect on the readings obtained. The effect of enzyme concentration on optical density measurement was tested by comparing a concentration of homogenate at 10 mg mites per ml buffer with one at 17 mg mites per ml buffer; in this instance enzyme concentration had no effect on the optical density readings.

A standard curve was determined for converting optical density values to micromoles of ACh; 0.015, 0.023, 0.03, 0.037, and 0.045-ml aliquots of 0.002 M ACh were made up to 0.3 ml in phosphate buffer pH 7.4, making final concentrations of ACh to range from 0.001 to 0.003 M. The optical density readings were plotted against the micromoles of ACh present in each sample (Fig. 9). The ChE activity expressed as micromoles of ACh hydrolysed was calculated by subtracting the micromoles of ACh remaining in the sample after incubation from the micromoles of ACh in the control sample without enzyme.

The percentage inhibition of the ChE was calculated for each malaoxon concentration and these values were plotted against the corresponding negative logarithm of the malaoxon concentrations. From the graphs obtained for the susceptible and OP-resistant strains the corresponding I_{50}'s were determined.

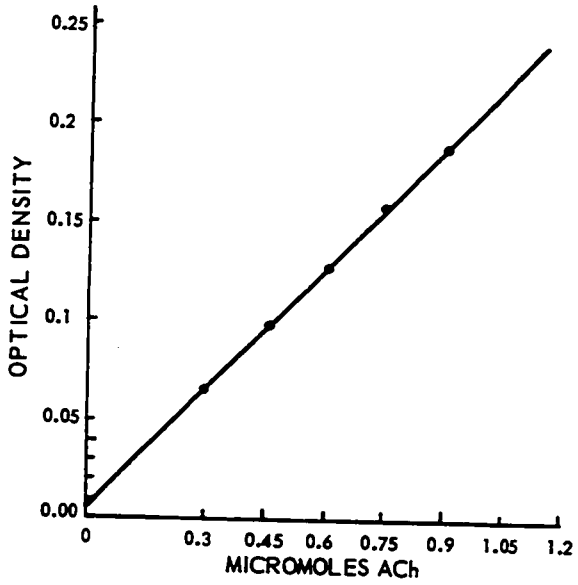


Fig. 9. Standard curve for ACh. Beckman DU spectrophotometer. 540 millimicrons. (light path at 1 cm).

RESULTS

1) Factors Affecting the Mortality Rate in the Toxicological Test

The variability within each set of 5 slides receiving each parathion dose on the same day was assessed by comparing the mean squares for the mortality percentages with the expected values for a binomial distribution. Since no evidence was found for a lack of homogeneity, there was no need to divide the day's sample of 50 mites at each dosage level among the 5 strips of tape and spray them separately. Therefore the results from each set of 5 slides were combined before the figures were subjected to probit analysis (Finney, 1952). Also in subsequent tests in this investigation all 50 females were put on one taped-slide and sprayed simultaneously. This finding is in disagreement with that of Dittrich (1962), although it is possible that his replicate sprayings were more widely spaced in time thus reflecting the between-test variation found in these experiments.

The statistical analysis of the dosage-mortality figures obtained with parathion revealed that the agreement with a linear regression of probit on log dose was good on the whole. The heterogeneity χ^2, used to test deviations from linearity, reached the 5% significance level in only one out of the 4 experiments. When the results from the four experiments were combined, the χ^2 for

heterogeneity was not significant.

The estimates obtained from the fitted regressions with the standard error and fiducial limits are shown in Table 1. As indicated, the between-assay variation in LD_{50} was significant, and thus this variation has been included when calculating weights and fiducial limits (Bliss, 1952).

i) Temperature

The pre-treatment temperatures had little effect on the resulting mortalities at two test concentrations of parathion (Table 2). The mortality rates from 2.74% parathion were identical whether the mites had been held for the preceding 24 hours at 7.2°C, 23.9°C, or at 26.7°C. With 5.38% parathion, at the upper end of the dosage-mortality relationship, there was some indication that higher pre-treatment temperatures reduced the mortality slightly.

The post-treatment temperature had a profound effect, in that the mortality was very much reduced at a low temperature (Table 3). Increase of post-treatment temperature in the higher range increased the mortality (Table 4). Under similar temperature conditions, the mortality was lower when the relative humidity was very high while at similar relative humidities it was greater at the higher temperature.

ii) Relative Humidity

With a graded series of post-treatment relative humidities derived from different saturated salt-solutions at a constant temperature (Fig. 10, Table 5), there was no discernible effect on the mortality over the range from 33% up to 96% R.H. But again there

Table 1. Statistical analysis of test method. Toxicity of parathion to the Niagara susceptible NS strain of T. urticae.

Slope	4.52
S.E.	0.320
LD_{50}	0.050
95% f.l.	0.043, 0.059
LD_{95}	0.115
95% f.l.	0.103, 0.128

All values based on data from four experiments, using weights inversely proportional to the estimated variances of the values from the separate experiments.

Table 2. Percentage mortalities of the NR strain treated with parathion after 24 hours at 3 different pre-treatment temperatures.

Pre-treatment Temperature	Concentration of Parathion		
	0%	2.74%	5.38%
26.7°C	0	62	82
23.9°C	0	61	92
7.2°C	0	64	96

50 adult females per treatment; held for 24 hours at 24°C and 95 - 100% R.H.

Table 3. Effect of post-treatment temperature and humidity on mortality of the NS strain treated with 0.050% parathion.

Temperature	Relative Humidity	Percentage Mortality
26.7°C	45 ± 5%	98
Control		0
23.9°C	42 ± 5%	100
Control		2
12°C	82 ± 5%	0
Control		0

Values based on 50 adult females per dose.

Table 4. Effect of temperature and humidity on mortality of the NS strain treated with 0.050% parathion.

Temperature (°C)		Relative Humidity	Percentage Mortality
Treatment	28.2 ± 5	58 ± 5%	98
Control			0
Treatment	24.5 ± 5	58 ± 5%	76
Control			2
Treatment	24.0 ± 5	95 - 100%	60
Control			0

Values based on 50 adult females per dose.

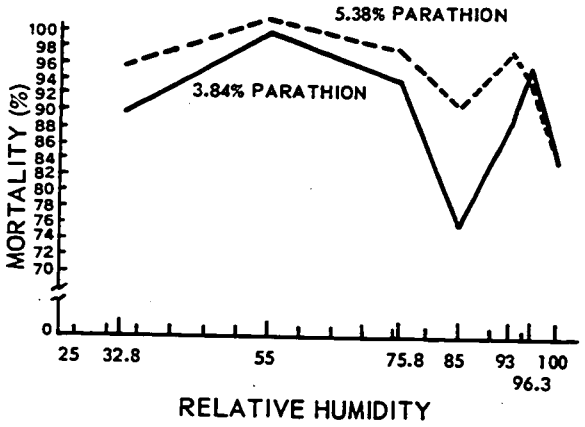


Fig. 10. Mortality of adult females of the NR strain at different humidities. Temperature constant at 23.9 ± 0.02°C.; control mortality 0-4%.

Table 5. Percentage mortalities of the NR strain treated with parathion and held for 24 hours at 7 different post-treatment humidities.

R.H.[a] (%)	Concentration of parathion		
	0%	3.84%	5.38%
100	0	84	84
96.3	0	96	94
93	0	89	98
85	0	76	93
75.8	0	94	96
55	0	98	100
32.8	0	90	96

[a]The respective humidities were obtained with the following saturated salt solutions:- R.H. 100% = distilled water; 96.3% = KH_2PO_4; 93% = KNO_3; 85% = KCl; 75.8% = NaCl; 55% = dextrose; 32.8% = $MgCl.6H_2O$.

Mortality values based on 50 adult females per treatment.

Post-treatment temperature 23.9 ± 0.02 C.

were indications that the mortalities are lower at the very high relative humidities close to 100% R.H.

iii) Age of Mites

Adult females a few hours old were found to be very susceptible to mevinphos (0.0018%) but females more than 2 days old showed negligible mortality at this dosage (Table 6); when about a day old they were intermediate in susceptibility (Table 6). With parathion (0.035%), females 2 - 4 days old showed 84% mortality, while those 7 - 15 days old showed only 60% mortality. With serial dosages of parathion (Table 7) females 31 days old were markedly less susceptible than those 14 days old, which were slightly less susceptible than those 7 days old.

iv) Time of Day

Mortalities at a single dosage of parathion (Table 8) did show a tendency to be lower at early morning (7:35 a.m.) and mid-afternoon (4:30 p.m.). The results were roughly the same with the resistant NR strain as with the susceptible NS strain (Fig. 11). Since there were some inconsistencies between the strains, a more detailed and replicated experiment conducted under more precisely controlled environmental conditions would be required to validate the peaks on the graph. At least the results indicate that the percentage mortalities can differ by as much as 30% in the median range according to the time of day when the test is conducted.

v) Viscosity of Spray Concentrations

The addition of 0.5% olive oil to the acetone solvent greatly increased the mortality of NR strain females from parathion solutions

Table 6. Relative toxicity of mevinphos (0.0018%) to adult NS-strain female mites of different ages.

Age	No. Tested	% Mortality
3.6 - 4.8 hrs.	496	71.0
14.4 - 26.4 hrs.	259	13.0
2 - 4 days	139	3.1
7 - 15 days	165	2.6
Check 7-15 days	50	0
Check 1-4 days	56	0

Table 7. Percentage mortalities of adult females of the NS strain treated with parathion at different ages.

% Concentration of Parathion	Age of females after final moult		
	7 days	14 days	31 days
0.1	100	98	94
0.07	98	94	84
0.05	96	90	70
0.035	88	82	66
0.025	74	60	48
0.013	32	30	18
Solvent Control	0	0	0
LD_{50}	0.017	0.021	0.028

Table 8. Effect of time of day of treatment on toxicity of parathion[a] to adult female T. urticae.

Time of treatment	NS strain			NR strain		
	Temp.°C[b]	R.H.	% Mort.	Temp.°C	R.H.	% Mort.
6:20 a.m.	29.5	64	96	24.8	78	52
7:35	28.5	70	80	25.2	78	48
8:30	28.5	68	92	27.1	74	76
9:30	29.4	69	92	28.8	78	72
10:30	30.0	69	88	29.4	69	52
12:30 p.m.	30.4	67	96	31.9	63	65
2:30	31.0	42	92	33.5	58	44
4:30	32.2	64	84	36.4	49	40
6:30 p.m.	32.0	68	84	32.2	64	52

[a]Dose for NS strain = 0.035%; for NR strain = 2.74%.

[b]Temperature of treatment and of post-treatment period.

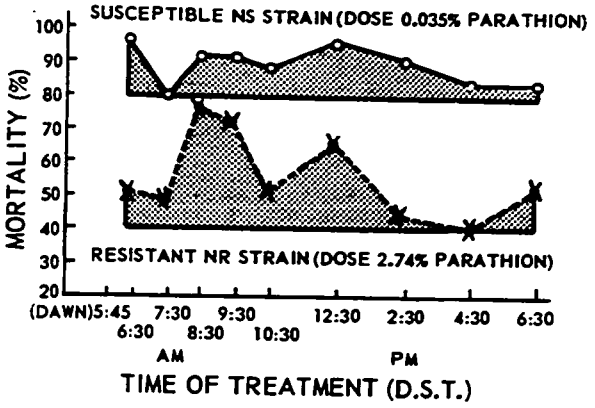


Fig. 11. Effect of time of day of treatment on the mortality of susceptible NS (circles), and parathion-resistant NR (crosses) strains.

(Table 9). The effect was most pronounced at low parathion concentrations, raising the mortality by 20 - 30% at parathion dosages up to 2.74%.

The addition of 0.9% corn oil to a 0.1% concentration of malathion and of 0.9% olive oil to a 0.1% parathion solution in order to increase their viscosity to that of the 1.0% concentrations of each toxicant (Table 10) showed that an increase in viscosity had the effect of increasing rather than reducing the mortality rate. Thus the reduction in mortalities observed at the higher concentrations of 1.0% of each toxicant, as compared to the 0.1% concentrations, cannot have been due to their increased viscosity.

vi) Point of Time at which Immobility becomes Permanent

With malathion it was found that the higher concentrations (e.g. 1.6%) gave lower 24-hour mortalities than low dosages (e.g. 0.2%) (Table 11). Mites treated at the higher dose exhibited an almost immediate partial paralysis and their leg movements were very slow, whereas females at the lower dose exhibited very rapid leg movements for approximately 4 hours after treatment and then usually stopped moving completely (Tables 12, 13). It appeared that the rapid leg movement of the lower dose served to distribute the poison to the active sites, whereas the immediate paralysis with the higher dose impeded the distribution of the toxicant. Thus 24 hours after the treatment more of the mites treated at the 1.6% dose were capable of some leg movement, albeit slow, than those treated at the lower dose, and this inverse difference also prevailed for the next 24 hours. Although eventually all mites treated at either

Table 9. Effect of the addition of olive oil (0.5%) to acetone solutions of parathion on the mortality of the NR strain.

% concentration of parathion	Percentage mortality	
	With oil	Without oil
7.53	98	88
5.38	92	90
3.84	84	76
2.74	80	57
1.96	47	27
Control (olive oil-acetone)	0	0

Values based on 50 adult females per dose.

Table 10. Percentage mortalities of the NS strain exposed to higher concentrations of malathion or parathion without oil and to lower concentrations to which oil was added to approximate the viscosity of the higher concentrations.

% concentration of toxicant	% concentration of oil	Percentage Mortality
Malathion	Corn oil	
0.1	0	74
0.1	0.9	86
1.0	0	64
0	0.9	4
0	0	2
Parathion	Olive oil	
0.1	0	86
0.1	0.9	92
1.0	0	74
0	0.9	6
0	0	0

Values based on 50 adult females per dose.

Table 11. Percentage mortalities of adult females of the NS strain exposed to higher and lower dosages of malathion. Counts made 24 hrs. after treatment.

Replicate	1.6% malathion	0.2% malathion
1	32	76
2	52	79
3	48	87
4	36	88
5	33	84
6	38	83
7	44	75
8	44	72
9	40	76
10		75
11		60
Mean*	40.7	77.7

* Difference between means significant at 0.1% level.

Table 12. Response of NS strain females to high and low malathion concentrations during the 72-hour period after treatment.

Hours after treatment	Numbers of mites showing 3 degrees of leg movement								
	Solvent only			0.2% malathion			1.6% malathion		
	Rapid	Slow	None	Rapid	Slow	None	Rapid	Slow	None
0.17	10	0	0	9	1	0	0	8	2
0.25	8	2	0	8	0	2	0	4	6
0.33	4	3	3	3	4	3	0	6	4
0.42	7	0	3	6	0	4	0	3	7
0.5	8	0	2	5	3	2	0	5	5
0.66	5	0	5	2	5	3	0	3	7
0.75	1	0	9	6	2	2	0	4	6
2.7	2	0	8	0	6	4	0	4	6
3.0	2	0	8	3	3	4	0	3	7
4.3	8	0	2	1	3	6	0	6	4
4.75	9	0	1	2	2	6	0	5	5
16.75	9	1	0	1	2	8	0	6	4
24.0	9	1	0	0	5	5[a]	0	7	3[a]
26.6	9	0	1	0	2	8	0	4	6
28.0	9	0	1	0	2	8	0	3	7
29.7	8	0	2	0	1	9	0	2	8
45.7	9	0	1	0	0	10[b]	0	1	9
48.0	9	1	0				0	4	6
50.3	7	2	0				0	2	8
72.0	9	1	0				0	0	10[b]

[a]percentage mortality for 0.2% dose after 24 hrs. = 50%; for 1.6% dose = 30%.

[b]100% mortality for 0.2% dose at 45.7 hr., for 1.6% dose at 72 hrs.

Table 13. Response of NS strain females to high and low malathion concentrations during the 24-hour period after treatment.

Hours after treatment	Numbers of mites showing 3 degrees of leg movement								
	Solvent only			0.2% malathion			1.6% malathion		
	Rapid	Slow	None	Rapid	Slow	None	Rapid	Slow	None
1	25	0	0	14	12	0	0	23	2
2.1	25	0	0	7	18	1	1	17	7
2.8	24	1	0	4	19	3	2	21	2
3.7	25	0	0	3	13	7	1	16	8
4.8	25	0	0	1	17	8	0	20	5
5.9	25	1	0	1	9	16	1	17	7
6.6	25	0	0	1	7	18	1	21	3
16.0	25	0	0	1	9	16	0	17	8
16.9	25	1	0	1	5	20	0	14	11
17.8	25	0	0	1	8	17	0	17	8
18.3	25	0	0	1	8	17	0	15	10
19.0	24	1	0	1	11	13	0	16	9
19.6	24	1	0	1	11	13	1	16	8
20.3	24	0	1	1	10	15	1	16	8
21.6	24	0	1	1	15	10	0	18	7
23.8	23	1	1	1	9	16	0	17	8
24.0	23	1	1	1	9	16[a]	0	19	6[a]

[a] Percentage mortality for 0.2% dose at 24 hrs. = 61.5%; for 1.6% dose = 24%.

concentration died when 72 hours had elapsed after the treatment, it remains true that at the 24-hour and 48-hour observation periods there were more individuals completely immobile at the lower dosage than at the higher. This phenomenon therefore results in a dip in the plateau region of the compound response curves for some compounds (Fig. 8).

2) Tests for Purity of the Susceptible Strain

i) Female Discriminating-Dose Test

The 50 lines reared from individual NS females, when tested by the female discriminating-dose test (Material and Methods p. 53), showed essentially the same response to various OP compounds as the original NS strain. For example, one of these selected strains, the NS_{21}, was compared with the NS strain in tests with Bidrin and phosphamidon; values for the LD_{50}, LD_{95} and the slope of the dosage-mortality line were found to be almost identical (Table 14).

ii) Male Discriminating-Dose Test

Males of the NS susceptible strain were all killed with a discriminating dose of 0.008% parathion (Table 15) whereas the mortality of resistant NR-strain males was only 20% as compared to a control mortality of 12 to 20 per cent. Strains reared from individual females chosen from the NS strain by the male discriminating-dose test gave responses similar to the NS strain when females were tested with serial dosages of ethion (Table 16). It may be therefore concluded that the NS strain is no different from lines pure for susceptibility bred from it by the male discriminating-dose test

Table 14. Relative toxicity of Bidrin and phosphamidon to the NS strain and the NS_{21} isolate of *T. urticae*.

Compound	Strain	Slope	S.E.	LD_{50}	LD_{95}	Ratio$_{50}$'s
Bidrin	NS	2.76	0.174	0.026	0.103	1.1
	NS_{21}	3.03	0.213	0.030	0.105	
Phosphamidon	NS	3.24	0.194	0.073	0.236	1.0
	NS_{21}	2.93	0.171	0.074	0.272	

Table 15. Relative toxicity of parathion to adult males of the NS and the NR strains.

% Concentration of parathion	Percentage mortality[a]	
	NS ♂	NR ♂
0.008	100	20
0.004	82	8
0.002	76	12
Solvent Control	20	12

[a] Based on average of 100 males per dose.

Table 16. Ethion dosage mortality values for six inbred lines (A - F) bred from isolated females selected from the susceptible NS strain and mated with a son.

Ethion (%)	Percentage mortality of strain[a]							
	A	B	C	D	E	F	NS_{21}	NR
0.03	92	74	94	86	76	95	92	1.3
0.02	88	68	96	82	52	92	88	0
0.014	42	32	90	58	44	74	83	0
0.0104	30	10	55	25	25	54	60	0
0.007	6	8	18	4	4	8	33	0
0.005	—	—	4	—	—	—	6	0

[a]Values based on data from two experiments for line E, all others based on three or more experiments.

Discriminating dose of parathion (0.008%) gave 100% mortality of F_1 male progeny of the parent female of each strain, except line F (96.6%).

Mortality of NR strain males at this dose was 0-20%.

and the female discriminating-dose test.

iii) Test of Progeny of Survivors

Of 331 females of the NS strain treated with parathion (0.002 to 0.032%) on leaf discs in petri dishes, only 21 were motile after 24 hours. Of these only two were fecund, laying a total of 7 eggs within 19 hours after transfer to clean leaves on wet cotton. Mortality of the mixture of the F_1 and F_2 females from these eggs was 100% and that of NS-strain females was 98% when treated at the discriminating dose of 0.34% parathion which kills almost all susceptible types.

Approximately 50 'live' females were removed without apparent injury from 20 different slides after being sprayed at 0.1% parathion. The dose caused 85 to 98% mortality. Only one of these females lived to oviposit on an isolated bean plant. The F_3 adult-female offspring from this survivor were placed on a taped-slide with NS strain females and treated with 0.1% parathion; the mortalities were 97% and 95% respectively. Mortalities of NR strain and NS strain untreated control females were 0% and 2% respectively.

Both above methods for obtaining 'survivors' had disadvantages. In the petri-dish method many of the mites wandered from the treated leaf discs onto the nylon screen and it was unlikely that all mites accumulated the same amount of toxicant. In the tape-spray method, it was extremely difficult to remove mites from the treated tape without injuring them.

Nevertheless, all the tests by the various methods indicated that the NS strain was pure for susceptibility, since both the lines

obtained from the individual females and the survivors of the discriminating doses gave mortalities or ld-p lines similar to those of the susceptible NS strain from which they came.

3) Inheritance of Parathion-Resistance

The ld-p lines obtained for response of the susceptible NS and the resistant NR Niagara strains to parathion (Fig. 12) are relatively steep, indicating that they are not heterogeneous. Moreover they are widely separated from each other, by 2 logarthmic cycles.

i) Reciprocal Crosses R♀ x S♂, S♀ x R♂

The F_1 females obtained from the reciprocal crosses between the S and R strains showed ld-p lines that were almost component to that of the R strain (Fig. 12) clearly indicating the almost complete dominance of resistance over susceptibility. There was no appreciable difference between the F_1 from the R♀ x S♂ cross and that from the S♀ x R♂ cross, indicating that resistance was transmitted by either sex and that there were no maternal effects.

ii) Cross of Intermediate Resistant Strain ♀ x R♂

The intermediate or I strain of the Niagara stock showed a stepped response curve for the females (Fig. 13a) indicating that it was a mixture of susceptible and resistant individuals. From the fact that the plateau was located at the 36% mortality level, it can be estimated by the Hardy-Weinberg law that this strain consisted of 36% ++, 48% R^+ and 16% RR females if a single dominant gene is involved and that thus the allele frequencies were 0.6 for + and 0.4 for R. All F_1 females obtained from the I♀ x R♂ cross were

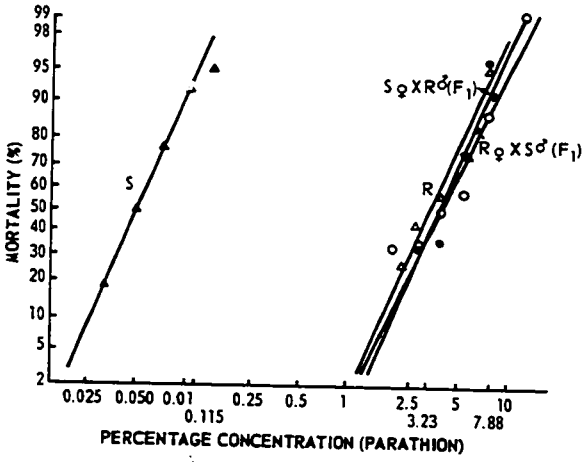


Fig. 12. Dosage-mortality lines for females of the NS and the NR (open triangles) strains and of the F_1 hybrid offspring between them. (R X S solid circles, S X R open circles).

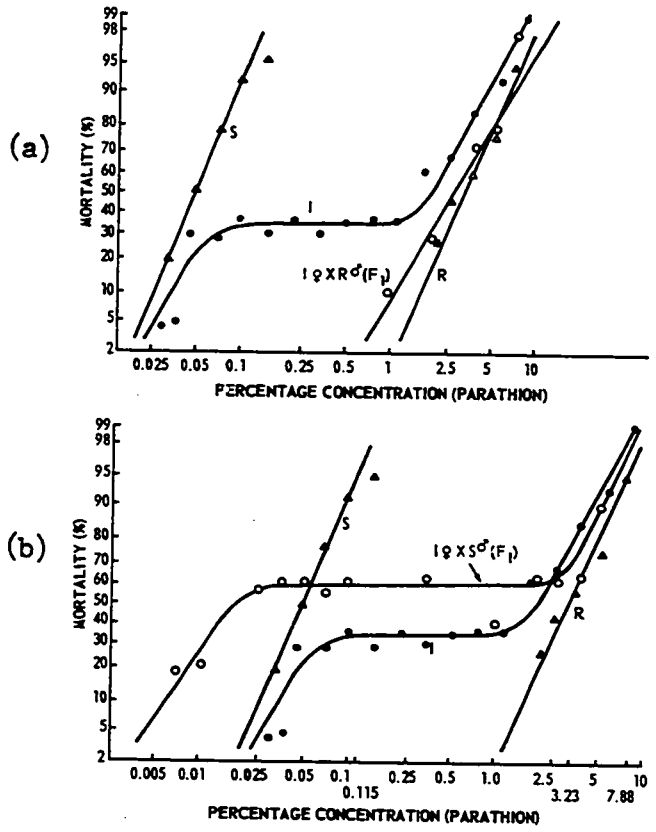


Fig. 13. Dosage-mortality lines for females of the partially resistant I strain and the offspring of a cross between females of the I strain and males of the NR strain (a), and males of the NS strain (b).

resistant (Fig. 13a), providing confirmation that the resistance factor is dominant and transmitted by either sex. Had the resistance been recessive the F_1 female progeny would have contained some 20% of +r susceptible types.

iii) Cross of Intermediate Resistant Strain ♀ x S♂

A cross of I♀ x S♂ would dilute the F_1 population with more ++ homozygous susceptible females. With an +-allele frequency of 0.6 in the I females and of 1.0 in the males of the S strain if it is pure, then the expected frequency of susceptible ++ homozygotes from the I♀ x S♂ cross will be 0.6. The offspring of this backcross (Fig. 13b) contained about 60% susceptible individuals since the plateau region of the curve is almost exactly at the 60% mortality level. This result provides evidence that allelism in a single major gene is responsible for the dominant parathion-resistance. Had resistance been recessive, all the females in the backcross offspring would have been susceptible.

iv) Backcrosses of F_1 Hybrids; (R♀ x S♂) x S♂, (S♀ x R♂) x S♂

The purpose of the backcrosses of the F_1 hybrids was to obtain additional proof of the monofactoriality of parathion-resistance in the segregation of the offspring. Such backcrosses should result in the female progeny consisting of two genotypes, resistant R^+ and susceptible ++, in a ratio of 1:1. The dosage-mortality results obtained for these backcross offspring did show a stepped regression line (Figs. 14a, b) but in each backcross the plateau was located slightly below the 50% mortality level, indicating a slight excess of resistant over susceptible females. However, the results do show

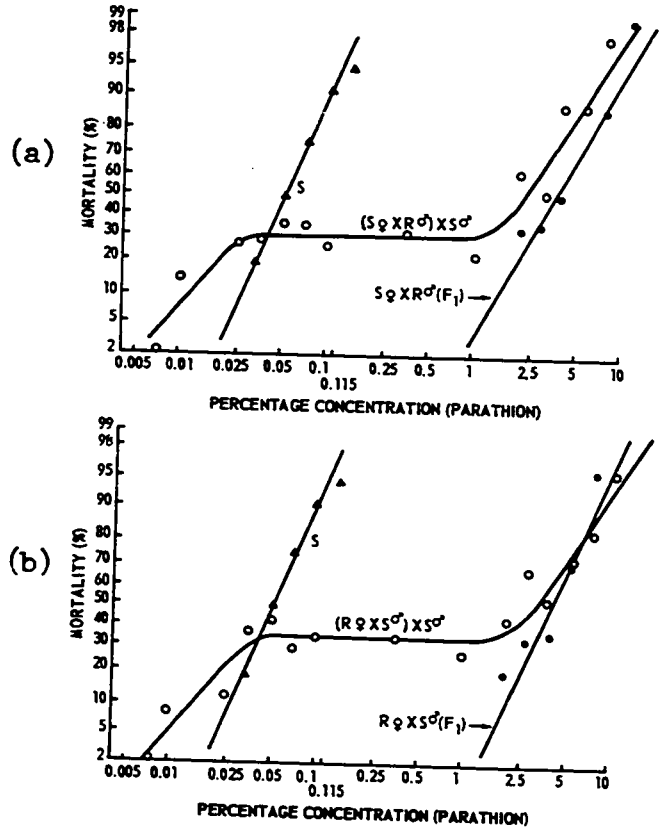


Fig. 14. Dosage-mortality lines for the female offspring of the backcrosses between (a) the F_1 hybrids from S females X R males, and (b) the F_1 hybrids from R females X S males, and males of the NS strain.

the sharp segregation of the backcross offspring into susceptible and resistant individuals which is strong evidence for monofactoriality in the inheritance of the dominant parathion-resistance.

Since the susceptible members of the backcross offspring were found to be more parathion-susceptible than their S-strain parents, this suggests that more eggs of the ++ genotypes failed to hatch than those with the R^+ genotype or that the ++ homozygotes might have survived in fewer numbers than the R^+ heterozygotes in proceeding from the egg-hatch to the adult stage.

4) Differences in Bionomics between Susceptible and Resistant Strains

Tests were made of possible bionomic differences in the S and R strains which could account for the greater survival value of the resistant genotypes indicated in the backcrosses previously described.

i) Fecundity and Egg-viability

It was found that there was no significant difference ($p > 0.05$) between the NS and NR strains in the average number of eggs laid over a 15-day oviposition period (Table 17). However, a significantly greater ($p < 0.05$) proportion of eggs were non-viable in the susceptible strain. This difference in egg-viability could account for the lower proportion of ++ females in the backcross offspring previously observed.

ii) Heat-hardiness

When females of the NS and NR strains were compared for their ability to withstand a 24-hour period at a graded series of 6

Table 17. Fecundity and egg-viability in the NS and NR strains.

Strain	No. of females	Cumulative no. of eggs per female				%
		2 days	3 days	9 days	15 days	Non-viable
NS	20	12.6	25	43.5	64.2	20.7
NR	20	12.7	28.9	47.6	68.2	2.5

Differences in fecundity not significant ($p > 0.05$).

Differences in egg-viability significant ($p < 0.05$).

temperatures above 40°C., it was found that the mortalities ranged from 3 to 10% lower for the susceptible strain than for the resistant one. However in this experiment, conducted at low R.H., the mortality did not increase in a regular way with increase in temperature. However, when the same tests were performed at near 100% R.H. linear regressions could be obtained for per cent mortality against the logarithm of the temperature (Fig. 15). It is seen that the lines of each strain are parallel to each other so that the LD_{50} for the NS strain was 41.3°C as compared with 40.7°C for the NR strain, and at the upper end of the line only the NS females survived 24 hours at 43°C. However, this interstrain difference is very small, and is not comparable to the definite reduced heat-hardiness observed by Dittrich (1961) in his resistant Leverkusen strain.

5) Mechanism of Resistance to Organophosphorus Compounds

The NS and NR strains were compared for the rate at which they degraded C^{14}-malathion and for the sensitivity of the ChE to malathion in order to determine whether the OP-resistance mechanism was increased detoxication or a less sensitive ChE.

i) Rate of Degradation of C^{14}-Malathion in Susceptible and Resistant Strains

The *in vitro* enzymatic degradation of C^{14}-malathion by homogenates (Table 18) revealed that the parathion-resistant NR strain had almost twice as much detoxicative capacity for malathion as the susceptible NS strain. Most of the malathion was hydrolysed at the carboxyester bond (Fig. 16), but there was also appreciable phosphatase activity. The percentages of carboxyesterase products

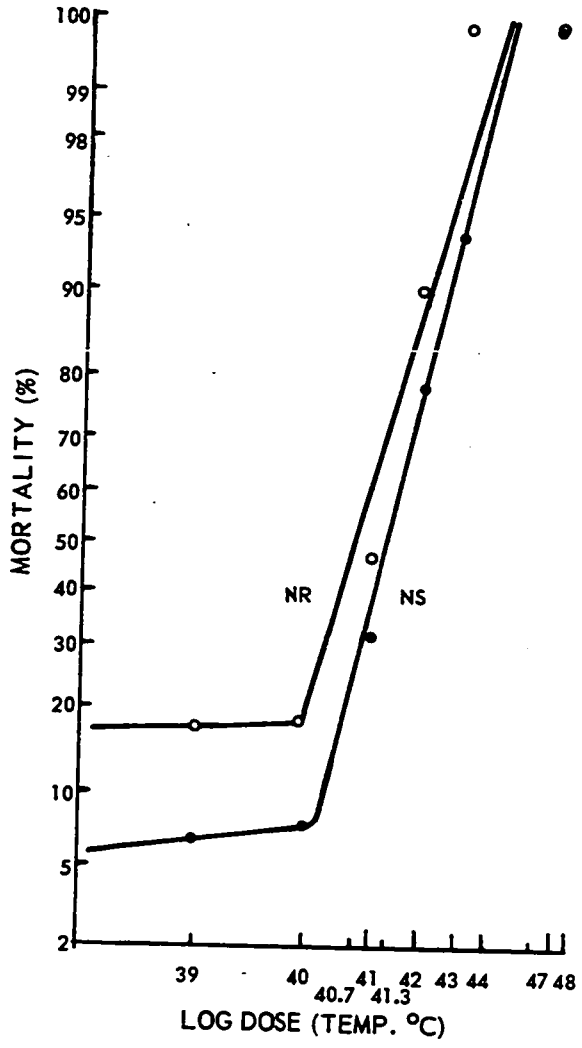


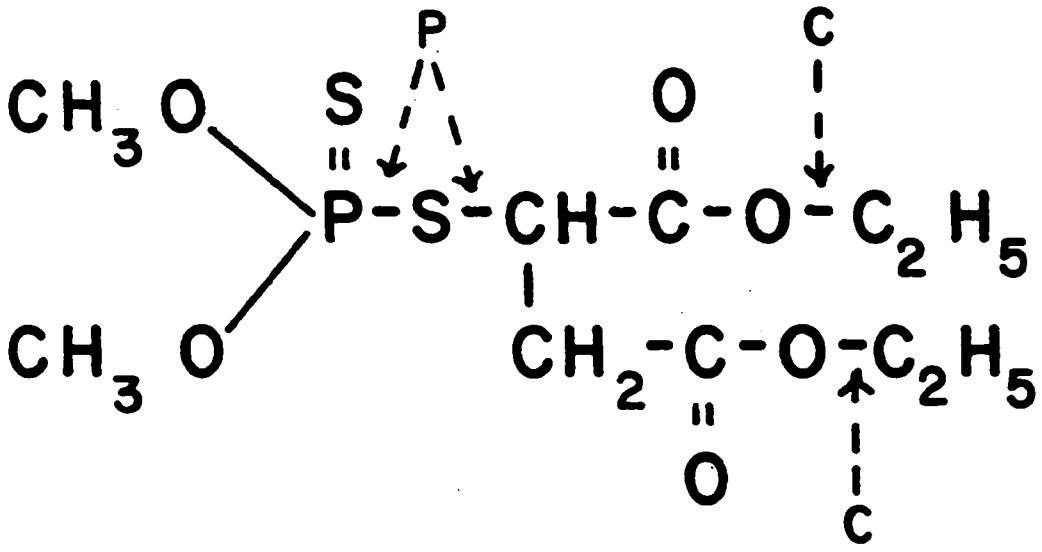
Fig. 15. Percentage mortalities of adult females of the NS strain (closed circles) and the NR strain (open circles) held for 24 hours at 6 different temperatures.

Table 18. Degradation of C^{14}-malathion by homogenates of the NS and NR strains *in vitro*.

	% of total activity added		% of total activity recovered	
	Susceptible	Resistant	Susceptible	Resistant
Carboxyesterase products	31.42	38.12	35.24	62.48
Phosphatase products	11.07	13.11	12.42	21.49
$CHCl_3$-soluble products[b]	46.66	9.78	52.34	16.03
Total	89.15	61.01	100.00	100.00

[a] Expressed in per cent of total malathion applied by incubation of a mite homogenate (20mg/ml) for 30 min. with 40 ug C^{14}-malathion in 0.01 ml ethanol per ml of homogenate.

[b] malathion + malaoxon + diethyl malate + diethyl mercaptosuccinate.



MALATHION

Fig. 16. Sites of enzymic hydrolysis of malathion (and malaoxon) by degradation enzymes.

P = phosphatase; C = carboxyesterase.

and phosphatase products produced were higher in the resistant strain whether the figures were calculated in terms of the amount of C^{14}-malathion applied or the amount recovered. The amount of toxicant remaining undegraded, as indicated by the chloroform-soluble fraction, was very much less in the homogenates from the resistant strains. The lower percentage of total activity recovered in the assay of the resistant strain may have been caused by slightly more mite debris and/or solute being trapped by the filter media. However, the percentage of activity recovered was still much higher than in comparable experiments by Matsumura and Voss (1964), probably due to the availability in these experiments of more sensitive scintillation-counting equipment and scintillation fluids. The radioactivity counts were almost identical for both the 'uncleaned' chloroform fraction and the column-cleaned sample indicating that quenching was negligible and therefore the cleanup procedure followed was unnecessary.

ii) Inhibition of Cholinesterase in Susceptible and Resistant Strains

When the cholinesterase activities of the susceptible and the parathion-resistant strains were assessed in vitro, they were found to be very similar (Table 19). From the very small amounts of enzyme source (0.24 ml of mite homogenate) and substrate (0.03 ml 0.002 M ACh) used to assess the activity, it could still be determined that the ChE activity of the Niagara strains were approximately 15% higher than that reported by Matsumura and Voss (1964) for their NN strain, which is the same as our NS strain. Optical density readings with 5 concentrations of malaoxon from 10^{-3} to 10^{-5} M

Table 19. Hydrolysis of acetylcholine by homogenates of the NS and NR strains.

Strain	μ moles ACh hydrolyzed/100 mg mites/hr.
Susceptible	10.25 ± 0.31
Resistant	10.00 ± 0.18

The enzyme extract was 2.4 mg/0.3 ml; substrate concentration 2×10^{-3}M; incubation temperature 30°C.

were similar, indicating the concentration of inhibitor or of acetone had no appreciable effect on the test results. The standard curve for ACh, used to convert optical density values to micromoles, was linear (Fig. 9).

When the cholinesterase enzymes of the two strains were submitted to graded concentrations of the inhibitor malaoxon (Fig. 17) it could be seen that the ChE of the parathion-resistant strain was no less sensitive than that of the susceptible strain. The percentage inhibition of ChE at inhibitor concentrations from 5×10^{-8} to 10^{-6} M ranged from 10.20 to 88.29% for the susceptible strain, and from 10.20 to 89.65% for the resistant strain. The cholinesterases of both strains were completely inhibited by malaoxon concentrations of 5×10^{-6} M and higher. The I_{50} concentration for malaoxon for both the Niagara strains was considerably lower than that reported for the NS strain by Voss and Matsumura (1965).

6) Cross-Resistance to OP Compounds in the Parathion-resistant Strain

i) Relative Toxicity to Susceptible and Resistant Females

When the Niagara NR strain, which had an 87-fold parathion resistance as a consequence of parathion selection, was tested for its cross-resistance to other OP compounds (Table 20), the resistance ratios ranged from about 3-fold for phosphamidon and Bidrin to 1000-fold for dimethoate. Arranged in order of increasing cross-resistance, the OP compounds were phosphamidon, Bidrin, malathion, fenthion, demeton, mevinphos, ethion, and dimethoate. The ld-p lines for the compounds tested all had steep slopes (averaging about

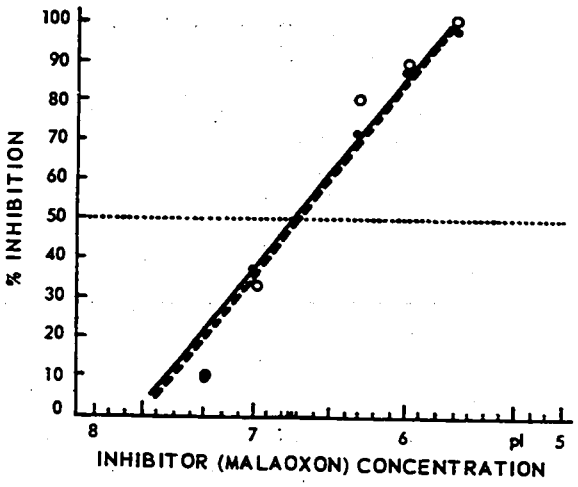


Fig. 17. Simultaneous inhibition of the ChE from the NS strain (circles) and the NR strain (crosses) by malaoxon inhibitor in the presence of ACh. pI = Negative logarithm of molar inhibitor concentration.

Table 20. Cross-resistance spectrum of the parathion-selected NR strain.

Compound	LC_{50}, per cent		Resistance Ratio
	Susc.	Resist.	
parathion (selecting agent)	0.037	3.20	87
demeton	0.0082	0.513	63
fenthion	0.041	1.88	46
malathion	0.095	2.90	30
ethion	0.0096	3.52	365
dimethoate	0.004	4.01	1010
mevinphos	0.0038	0.570	150
Bidrin	0.026	0.057	+2
phosphamidon	0.074	0.130	+2
dicofol (non-phosphate)	0.032	0.032	1

3.0) with both the susceptible and resistant strains, but the LD_{50}'s varied much more with the susceptible strain than with the resistant one (Figs. 18,19,20). It was the compounds dimethoate, ethion, mevinphos and demeton, which were the most toxic (LD_{50}'s $<0.01\%$) to the susceptible strain, and it was to these that the parathion-resistant strain showed the highest levels of cross-resistance. Conversely it was those compounds which were the least toxic to the susceptible strain, namely Bidrin, phosphamidon, and malathion, to which the lowest cross-resistance was shown by the NR strain. The parathion-selected NR strain had developed no cross-resistance to the chlorinated acaricide dicofol (Fig. 19d).

When the cross-resistance to the carbamoyl oxime Temik was tested in the NR strain by means of granules applied to the soil of potted plants, it was found that there was a low level of cross-resistance. At the rate of 0.023 gm actual per 4-in. pot, mortalities were 100% at 48 hours after treatment for both strains. With Temik applied at the rate of 0.0115 gm toxicant per pot, the 48-hour mortality was 49% for the NR strain as against 100% for the susceptible NS strain. The 72-hour mortality at this dosage was 100% in both strains, as also was the 48-hour mortality at double this dosage.

7) Cross-Resistance to OP Compounds in Bidrin-selected Strains

i) The Bidrin-selected NS Strain

After the Niagara susceptible strain had been selected for 10 generations with Bidrin, the resulting NBR strain was tested for cross-resistance (Table 21; Fig. 20a,c,d,). Although only a 3-fold

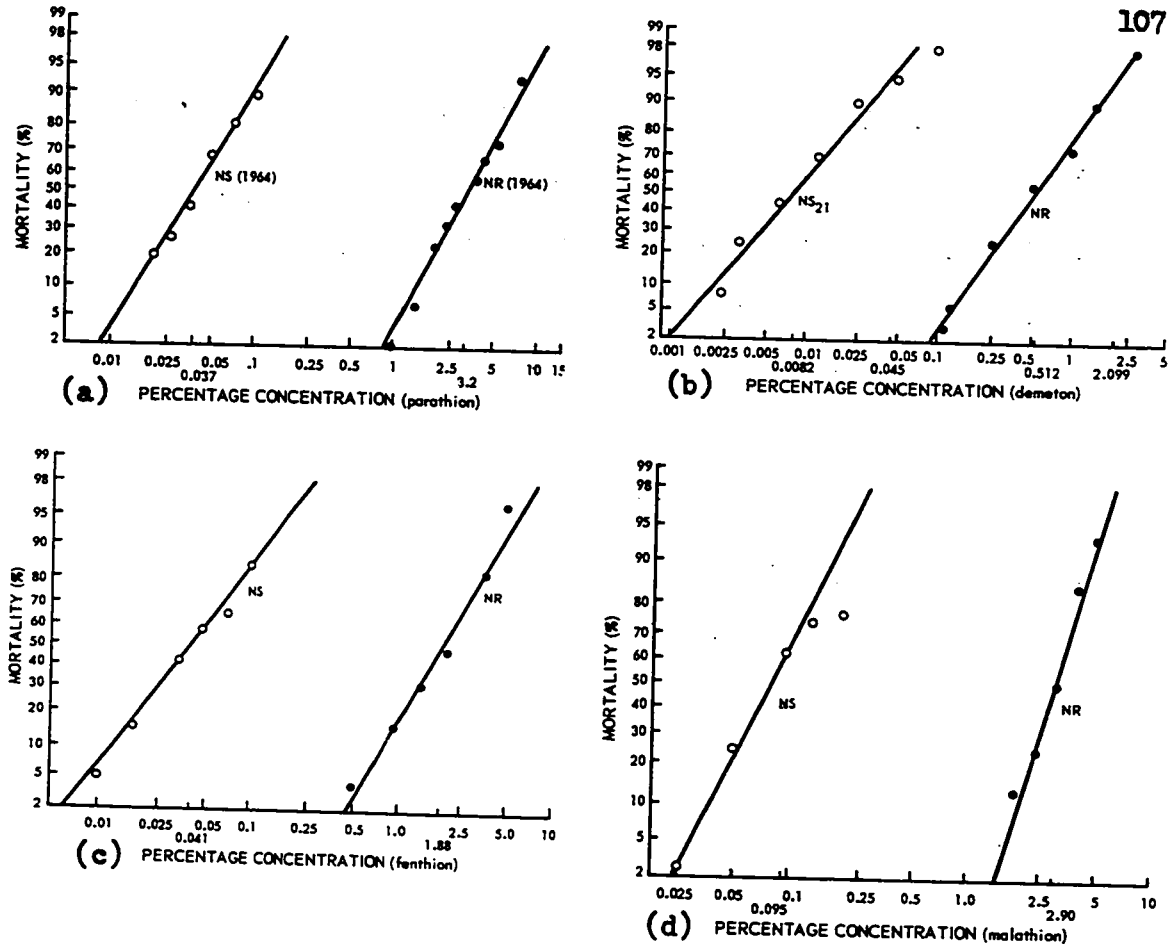


Fig. 18. Dosage-mortality relationships to (a) parathion, (b) demeton, (c) fenthion, (d) malathion for the susceptible NS strain and the parathion-selected NR strain.

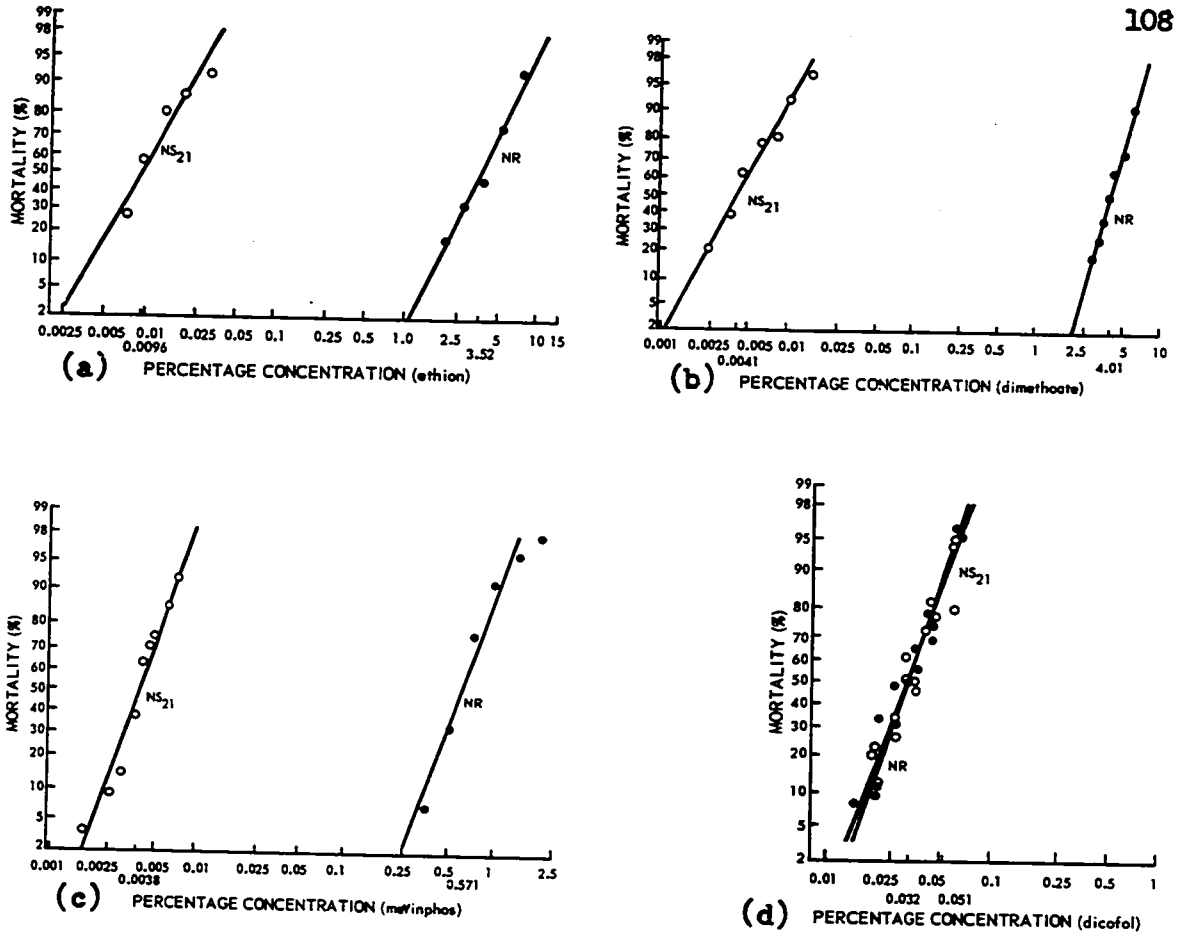


Fig. 19. Dosage-mortality relationships to (a) ethion, (b) dimethoate, (c) mevinphos, (d) dicofol for the susceptible NS strain and the parathion-selected NR strain.

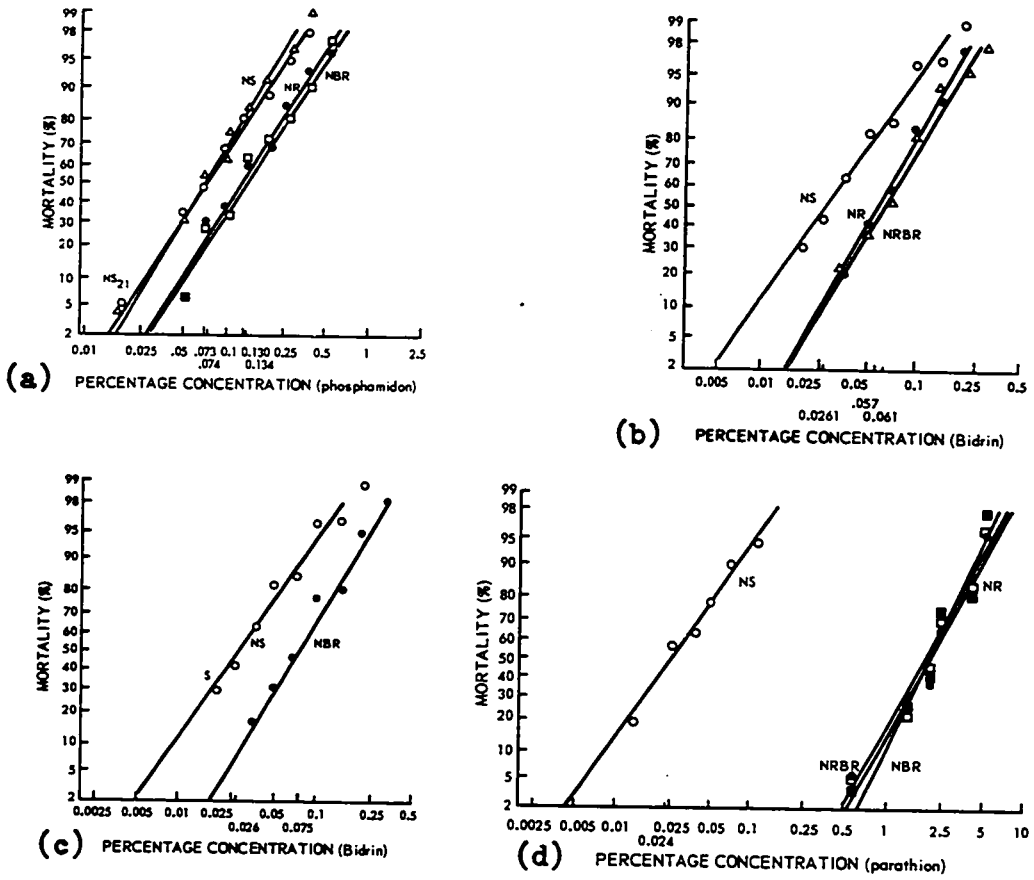


Fig. 20. Dosage-mortality relationship to (a) phosphamidon, (b) Bidrin, (c) Bidrin, (d) parathion for the NR, NBR, or NRBR strains.

Table 21. Cross-resistance spectrum of the Bidrin-selected NBR strain.

	LC50 per cent		
Compound	Susc.	Resist.	Resistance Ratio
Bidrin	0.026	0.075	3
phosphamidon	0.073	0.134	2
parathion	0.024	1.967	80

resistance to Bidrin had been developed, and a 2-fold cross-resistance to phosphamidon, the cross-resistance developed to parathion was fully 80-fold. These resistance levels in the Bidrin-selected NBR strain are almost identical with those developed by the parathion-selected NR strain to these 3 compounds (Table 20; Fig. 20a,b,d). Thus parathion and Bidrin each selected for the same level of cross-resistance to phosphamidon, and Bidrin selects for as high a resistance to parathion and as low a resistance to itself as parathion does.

ii) The Bidrin-selected NR Strain

Further selection of the parathion-resistant NR strain with Bidrin for 10 generations was found to have increased neither the cross-resistance to Bidrin nor the resistance to parathion (Table 22; Fig. 20b,d). Evidently the fullest resistance to these compounds had already been developed in the NR strain, so that further selection could not even increase the resistance ratio to parathion.

Table 22. Cross-resistance spectrum of the Bidrin-selected NRBR substrain of the parathion-selected NR strain.

Compound	LC_{50} per cent		Resistance Ratio
	Susc.	Resist.	
Bidrin	0.026	0.061	2
parathion	0.024	1.92	80

Table 22. Cross-resistance spectrum of the Bidrin-selected NRBR substrain of the parathion-selected NR strain.

Compound	LC_{50} per cent		Resistance Ratio
	Susc.	Resist.	
Bidrin	0.026	0.061	2
parathion	0.024	1.92	80

DISCUSSION

The genetic crosses in this investigation have proved that the inheritance of parathion-resistance in the parathion-selected Niagara strain of T. urticae is based mainly on a single dominant gene allele transmitted by either sex, and without maternal effects. These results agree with those obtained by Taylor and Smith (1956), Helle (1962) and Schulten (1966) with T. urticae, and with those of Andres and Prout (1960) with T. pacificus. These results do not agree with those of Dittrich (1961, 1963 a,b), who concluded that the inheritance of OP-resistance in T. urticae was polyfactorial, involving semi-lethal genes making for inviability when homozygous, plus a recessive factor also semi-lethal when homozygous. However, The conclusions of Dittrich regarding the recessive factor were simply based on certain assumptions regarding the rate of loss of the + allele under selection pressure as already discussed in the literature review, and not on the phenotype of the F_1 hybrid from crosses between resistant and susceptible strains which is the accepted method of determining whether a factor is dominant or recessive. Helle (personal communication 1966) concluded from his own work and that of Schulten (1966) on the Leverkusen SP strain homozygous for the resistance factor, that "all conclusions and speculations of Dittrich turned out to be erroneous". The depressed vitality found by Dittrich in his highly-selected Leverkusen RR strain was

probably caused by the heterogeneous nature of his strain which showed a very low slope value (about 1) since no depressed vitality was found in the homozygous, and stable Niagara resistant NR strain nor in the homozygous SP strain of Helle (1962). Therefore any factors depressing vitality cannot be a pleiotropic expression of the major resistance gene, although as stated by Helle (1965a) they may possibly be linked with minor resistance factors. The results obtained by most investigators with T. urticae and of Andres and Prout (1960) with a strain of T. pacificus agree in indicating that the inheritance of OP-resistance is probably monofactorial and that the resistance is dominant in all tetranychid mites. The results of the present investigation with the partially-resistant I strain containing both susceptible and resistant mites have been such as to provide additional proof of the dominance and monofactoriality of the main parathion-resistance factor.

The various tests for purity applied to the Niagara NS strain determined conclusively it was essentially pure, despite the dip in the response curve (Fig. 8). This NS strain could not have entirely lacked a certain number of heterozygotes or it would not have been possible to select the NR or I strains from it. However, the small percentage of non-susceptible individuals in this strain has not increased measurably since the colony was first established, as indicated by the test results obtained on the NS strain over a 4-year period as compared with those of the parent colony at Middleport, N. Y. The tests of the effect of viscosity on mortality, and of the point of time at which immobility of malathion-treated mites becomes permanent, failed to show the exact cause of the dip in the plateau region of the

dosage response curve; but the results with malathion, ethion and dimethoate did prove that this phenomenon is not an artifact. For the dosage-mortality relationship between parathion and T. pacificus, Andres and Prout (1960) figured a compound response line with a level plateau region; but had they joined the individual points for mortality they would have derived a curved dip in the plateau region almost identical with that found in this study, although they used a dipping method with water emulsions instead of a spraying method with acetone solutions. Schatz et al. (1964) have reported numerous examples of such "paradoxical effects whereby toxicity first increases then decreases as the concentration of toxicant increases". Hoskins (1963) stated that "many writers have drawn straight lines, though their data indicated otherwise, under the impression that departures from linearity were due to experimental errors".

To measure the degree of resistance to pesticides in arthropod populations, and to determine the genotype with respect to resistance, a log-dosage probit-mortality regression line (ld-p line) is obtained by treating representative samples with serial doses of toxicant on a logarithmic scale (Finney, 1952). With a sample homogeneous for either resistance or susceptibility, a straight regression line results when the percentages of mortality transferred to probits are plotted against the logarithm of the dose, as found with parathion in the NS strain (Fig. 12). However, as pointed out by Hoskins (1960), the ld-p line is only "approximately straight over at least the central mortality range, say from 20 to 80% and often farther". A slight curve above 80% mortality commonly occurs, especially when the toxicant does

not reach the active site at concentrations proportional to the dose applied, or when the toxicant contains impurities of different biological activity (Hoskins 1963, Georghiou 1965). When a population contains both resistant and susceptible genotypes, a compound response curve results (Tsukamoto, 1963); this was found with the I strain (Fig. 13). But if only a few serial dosages are used in testing a mixed population, wide intervals between doses may not reveal the compound nature of the curve, and a straight line may be drawn erroneously indicating a homogeneous population (Davidson 1960, Hoskins 1960). It is apparent that an accurate and detailed toxicological test is essential to measure resistance and interpret the inheritance of resistance in a population. The available test methods for establishing levels of susceptibility and of resistance in insects and mites have been reviewed by Quarterman (1960) and Reynolds (1960, 1962).

In studies of resistance and its inheritance in mites, some workers have used spraying methods (Saba 1961b, Dittrich 1961), while others have employed dipping methods (Andres and Prout 1960, Helle 1962) to apply the toxicants. Dittrich (1962) compared 4 toxicological test methods with T. urticae and concluded that the dipping method was the most sensitive requiring a lower dosage range but that the 'cage spray' method involved less variability. Dittrich maintained that at each dose at least 10 replicates of up to 60 mites should be tested to provide an estimate of the variances for each dose, since he found a "substantial batch to batch" variation. The statistical analysis of the method in this study showed no evidence for a lack of homogeneity when testing the variability within each set of 5 slides receiving each

parathion dose. Dittrich did not use the Potter tower, which is widely known to be an accurate spray device (Harris et al. 1962) in his spray test. Moreover, his replicate treatments were probably more widely separated in time than ours, thus adding to the variance.

Helle (1962) could not obtain an ld-p line for his OP-resistant strain by his dipping method because concentrations greater than 6000 ppm parathion were phytotoxic. He also reported the dipping method to involve "considerable variations in the slope value b and the LD_{50} in the various experiments". In the present investigation it was essential to obtain an ld-p line for parathion with the Niagara parathion-selected strain and therefore a new, accurate and less sensitive test method was developed.

The assessment of the effect of various factors on the toxicity of the OP compound DDVP made by Kensler (1965) with a fumigation test method on a susceptible strain of T. urticae, is in general agreement with those in this investigation; the only exception was that he reported that differences in post-treatment temperature and in relative humidity had little effect. Kensler found that mortalities were similar at low temperature and relative humidity (70°F, 35% R.H.) as at high temperature and relative humidity (90°F, 88% R.H.). Such results are in agreement with those in this investigation where mortalities were found to be higher at lower relative humidities when the temperature was constant. Had Kensler compared mortalities at 70°F and at 90°F with relative humidity constant he would likely have found that mortalities were higher at the 90°F temperature. Harrison and Smith (1961) found that the toxicity of dicofol to eggs of T. urticae

increased as the relative humidity was decreased from 60% to dryness. The increase in mortality at very high relative humidities from 95% to 100% found by these authors, which is contrary to the results obtained in this study was probably due to the prolonged period of exposure, of as much as 7 days, of the eggs to these very high humidities. In agreement with Kensler's results, they found that different pre-treatment temperatures had little effect, and that the susceptibility decreased with an increase in the age of the mites; both of these conclusions are also indicated in this investigation.

That the time of day when a test is conducted has an effect on the per cent mortality of mites is now well established (Polcik et al. 1964, Kensler 1965, Fisher 1967), although there is no agreement as to when the mortality is at its peak. This inconsistency could be caused by the difference in test methods and in the insecticide used in the various investigations. Previously the factor of time of day was not considered to be a critical variable in toxicological tests (Busvine, 1957). The results with parathion in the NS and NR strains of T. urticae in this investigation do at least point to the need to standardize this factor in making susceptibility tests.

There is ample evidence that two mechanisms of OP-resistance occur in tetranychid mites. In the Niagara strain of T. urticae used in this investigation and in the Blauvelt strain used by Matsumura and Voss (1964, 1965), the mechanism was found to be that of detoxication. On the contrary the work of Voss and Matsumura (1964) and of Smissaert (1964, 1965) showed that in the Leverkusen strain of T. urticae the resistance mechanism is a cholinesterase enzyme insensitive to

inhibition by OP compounds and carbamates. There was also some evidence that OP degrading esterases are slightly more active in the Leverkusen resistant strain so that detoxication may also contribute slightly to the OP-resistance. The Niagara NR strain in the present investigation proved to hydrolyse most of the malathion and/or malaoxon at the carboxyester bond, which is in agreement with the results obtained by Matsumura and Voss (1964) with the Blauvelt parathion-resistant strain. The increased hydrolysis at the phosphoroester bond found for the NR strain in these experiments also agrees with their results. They found further interstrain differences; the rate of ChE inhibition *in vivo* by malathion was lower in the Blauvelt resistant strain than in the Niagara susceptible strain, and that the resistant strain had a slightly lower hydrolytic activity on α-naphthylacetate, thus indicating that there might have been some conversion of aliesterase to OP-detoxifying esterases. However, as both Smissaert (1965) and Matsumura and Voss (1964) were of the opinion that in mites these modified esterases, which were also found in the Leverkusen SP strain, are not major degradation enzymes.

No difference was observed in this study between the resistant and susceptible Niagara strains in their ChE activity against ACh substrate, in contrast to the finding of Matsumura and Voss (1965) that the ChE activity of the Leverkusen resistant strain was much lower than that of the Leverkusen susceptible strain. Also Yamamoto and Nishida (1961) found that there was little difference in the cholinesterase activity of a strain of *P. citri* 134-fold resistant to parathion and of a susceptible strain, indicating that the mechanism of resistance

in this strain, may also be that of detoxication.

Voss and Matsumura (1964) had found that the carbamate Temik (UC 21149), a potent ChE inhibitor did not control the Leverkusen RR strain which was characterized by an insensitive ChE, but it did control the Blauvelt R strain which had an enhanced detoxicative resistance mechanism. This evidence was presented as confirmation of the difference in the resistance mechanisms between these two strains. In this investigation Temik was also effective against the Niagara parathion-resistant strain despite encountering a slight cross-tolerance; thus the Niagara strain resembles the Blauvelt strain not only in detoxicative ability but also in being controllable by Temik. In this study the values of I_{50} (concentration of inhibitor required to inhibit 50% of the enzyme) for the ChE of the Niagara NR homogenate to malaoxon was 6.73, very similar to that of 6.75 found for the NS strain ChE. This is in contrast to the I_{50} concentration for the Leverkusen resistant strain in which the I_{50} was 2 points greater (i.e. 100-fold smaller) than that of the Leverkusen susceptible strain (Voss and Matsumura, 1965). Thus the Niagara parathion-resistant strain is characterized not by an insensitive ChE but by increased detoxication.

The Leverkusen resistant strain, used as the SP strain by Helle (1962) and as the R strain by Dittrich (1961), had been developed by selection with demeton and oxydemetonmethyl, respectively; on the other hand, the Blauvelt resistant strain and the Niagara NR strain had been selected with parathion. The original Cranbury strain of Taylor and Smith (1956), obtained from a greenhouse at Cranbury, Massachusetts, where it had been treated with parathion and/or TEPP

because demeton was not yet in common use, has been reported to be controlled by Temik (Weiden et al. 1965), and thus probably is characterized by detoxication rather than ChE insensitivity. It is therefore tempting to speculate that demeton or its analogues select for the resistance mechanism of an insensitive ChE, and that parathion selects for the resistance mechanism of detoxication. Parathion was one of the first OP compounds used extensively in the orchards of North America and elsewhere, and tetranychid mites generally had already developed this type of OP-resistance before demeton was introduced (Garman 1950, Cutright 1956, Glass 1960, Herne 1962, Jeppson and Jesser 1962). This would account for the fact that the carbamate Temik, like Bidrin and Azodrin, has been widely reported to be effective against OP-resistant tetranychid mites (Weiden et al. 1960, Allen et al. 1964, Howitt personal communication, 1967).

The resistance to parathion in the NR strain was very stable, there being no reversion toward susceptibility after a 9-month period without selection pressure, and the slope of the ld-p lines were steep to all compounds tested. This agrees with the results of Helle (1965a) with the SP strain. The rapid reversion to susceptibility of the OP-resistant strains of Saba (1961) and Dittrich (1961) could have been due to the heterogeneity and lack of stability reported for their strains. The straight ld-p lines with low slope values figured by these authors (Dittrich, 1963b) are more probably compound response curves with relatively steep slopes for each of the 2 or 3 genotypes of which they are composed.

Certain authors (Garman 1950, Dittrich 1961, Saba 1961a) have re-

ported OP-resistant strains of T. urticae to revert to susceptibility within a year after the removal of selection pressure. Yet the heterogeneous and partially-resistant I strain of this investigation became almost completely resistant after a period of 7 months without any selection whatever (Appendix 2; Fig. 3). Evidently the resistant genotypes in this partially-resistant strain must have had some bionomic advantage, perhaps a superior egg viability, over the susceptible genotypes.

Neither the parathion-selected NR strain of this investigation nor the demeton-selected Leverkusen strain used by Helle (1962), showed any cross-resistance to the chlorinated acaricide dicofol. A parathion-selected strain has been reported by Hansen et al. (1963) to be cross-resistant to chlorinated acaricides, but this strain had been initially very heterogeneous as attested by the low slope of its ld-p lines and had been taken from a "wild population" already partially resistant to malathion and parathion. The Leverkusen and NR strains, on the other hand, had steep slope values and were thus very homogeneous. In no instance has cross-resistance to chlorinated acaricides been reported in OP-resistant strains that had been selected with organophosphates from susceptible strains not previously exposed to OP compounds or chlorinated acaricides.

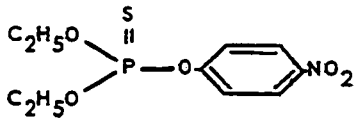
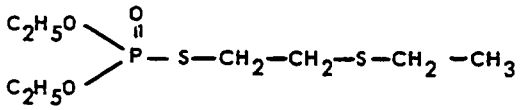
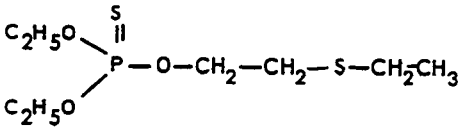
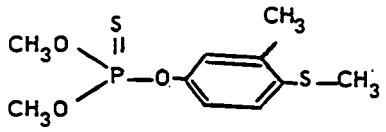
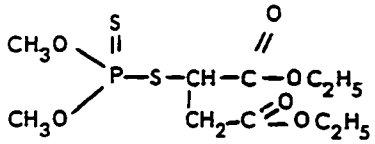
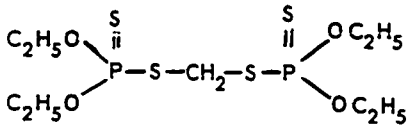
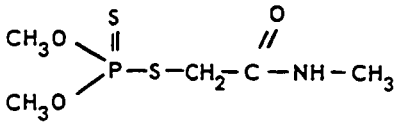
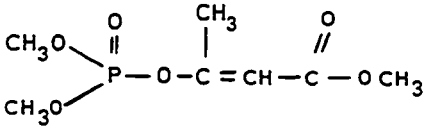
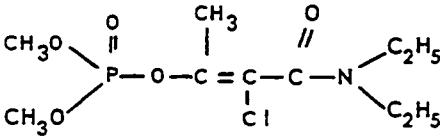
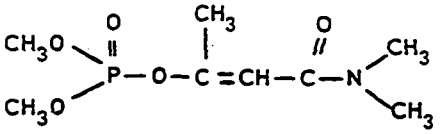
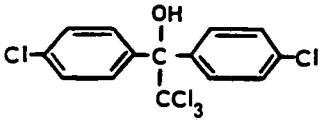
Hansen et al. (1963) found that selection of their T. urticae strain with parathion resulted in a higher level of cross-resistance to malathion than the resistance it selected to itself. In this investigation it was the opposite, the cross-resistance to malathion being less than half the resistance to parathion. However, as mentioned,

their parent susceptible strain, before selection, showed a low level of resistance to parathion and a slightly higher resistance to malathion.

The two mechanisms of OP resistance in tetranychid mites complicate the cross-resistance situation and probably have contributed to the diversity of results reported by different investigators. For example, a very low cross-resistance to Bidrin and phosphamidon was found in the parathion-selected NR strain in these experiments, a finding similar to that of others (e.g. Allen et al. 1964); whereas OP-resistant populations of P. ulmi have been found to be still controllable by Azodrin, an analogue of Bidrin (Howitt 1967, personal communication). However, Dittrich (1966) reported high levels of cross-resistance to Bidrin and phosphamidon in an OP-resistant strain of the carmine mite T. telarius in Switzerland, although he used a susceptible strain of T. urticae as his normal standard of comparison. The mechanism of resistance in the OP-resistant strain of the carmine mite may have been an insensitive ChE, but the selecting agent or agents were apparently unknown. The use of 2 species in Dittrich's cross-resistance study may also have clouded his results since Jeppson et al. (1964, 1965) found major interspecies differences in the relative susceptibility levels of P. citri and T. pacificus to different OP compounds. In the Niagara strain selected with parathion, an ethyl-substituted compound, the cross-resistance was nevertheless quite high to the methyl-substituted compounds malathion, fenthion, mevinphos and dimethoate (Table 23; Fig. 18 a,c,d; Fig. 19 b,c). In general, however, the cross-resistance was less to the methyl than to the ethyl compounds.

The cross-resistance in the parathion-selected strain was high to

Table 23. Formulae of compounds used in cross-resistance study.

COMPOUND	FORMULA	RATIO $LD_{50}NR / LD_{50}NS$
parathion		87
demeton	 + 	63
fenthion		46
malathion		30
ethion		365
dimethoate		1010
mevinphos		150
phosphamidon		2
Bidrin ®		3
dicofol		1

compounds with the phosphate group (mevinphos), the phosphorothioate group (demeton, fenthion), and the phosphorodithioate group (ethion, dimethoate).

The parathion-selected strain was highly cross-resistant to mevinphos which has a carbomethoxy ($-\overset{O}{\overset{\|}{C}}-O-CH_3$) group, but not to phosphamidon or Bidrin which have a diethyl or dimethyl carbamoyl group ($-\overset{O}{\overset{\|}{C}}-N-(C_2H_5)_2$ or $(CH_3)_2$) respectively (Table 23). The carbomethoxy group was thus associated with high toxicity to susceptible mites ($LD_{50} < 0.01\%$) whereas the carbamoyl compounds suffered very little from cross-resistance in the parathion-selected strain.

The carbamoyl compounds with the phosphate grouping, phosphamidon and Bidrin, suffered least from cross-resistance in the parathion-selected strain; whereas dimethoate, a carbamoyl compound with a phosphorodithioate grouping, showed the highest level of cross-resistance (Table 23). Dimethoate was highly toxic (LD_{50} 0.004%) to the susceptible strain but also suffered from a high level of cross-resistance in the parathion-selected strain (LD_{50} 4.01%). However, dimethoate differs from the carbamoyl phosphates not only in being a phosphorodithioate but also in lacking an unsaturated bond, and it differs from the phosphate Bidrin in having only one methyl group attached to the carbamoyl nitrogen.

Selection of the Niagara strain with Bidrin produced a high level of apparently stable resistance in only twice the number of generations that it took to obtain the partially-resistant I strain by selection with parathion. This may have been because the Bidrin selection was carried out at the 95-99% mortality level whereas the parathion

selection pressure for the I strain was carried out at the 80% mortality level. Indeed Watson and Naegele (1960) found that resistance developed faster in T. urticae when a high selection pressure was employed.

In this investigation Bidrin and parathion each selected for the same levels of cross-resistance to phosphamidon, and Bidrin selected for as high a resistance to parathion and as low a resistance to itself as parathion did. Therefore, it does not necessarily follow that the results obtained by previous workers (Hansen et al. 1963, Jeppson 1963, Georghiou and Bowen 1966) from selecting strains of T. urticae, T. pacificus, and the housefly respectively, with different OP compounds indicate only differences in the cross-resistance patterns. Here is an example in the Niagara stock of T. urticae of a similar cross-resistance pattern being induced by two different OP compounds.

It was found that a subsequent selection of the parathion-resistant NR strain with Bidrin for 10 generations did not further increase the resistance level to either Bidrin or parathion. There is no precedent in the literature to indicate that the cross-resistance induced by selection with one OP compound cannot be changed by further selection with another. It is possible that the homozygosity for resistance attained by the original selection with parathion had already established the fullest cross-resistance levels to the other OP compounds. This is not to say that selection of the NR strain with Bidrin for a further number of generations might not have ultimately increased the levels of cross-resistance induced by selection with parathion, especially if a second mechanism of resistance were to be

added to the first.

The results obtained with the parathion-selected Niagara strain of T. urticae confirm that certain OP compounds possessing a carbamoyl oxime group plus a phosphate group, and some carbamates possessing a carbamoyl oxime group are not handicapped by any considerable levels of cross-resistance being shown to them by the OP-resistant tetranychids. Some of the carbamoyloxy phosphorodithioate compounds also have been found by Jeppson et al. (1966) to suffer very little cross-resistance in strains of T. pacificus and P. citri made resistant by selection with ethion, and demeton plus parathion respectively.

Jeppson et al. (1966) have suggested that the search should be made for OP compounds to which susceptible strains could not develop resistance or only very slowly, and those compounds to which susceptible mites could rapidly develop resistance, such as the phosphoramidothioate compounds (e.g. Dowco 133), should be dropped from investigation. The present study indicates that in T. urticae it is possible to start with a fully OP-resistant strain as a base, and to employ OP compounds such as Bidrin to which there is virtually no cross-resistance. However, only time will tell whether these cross-resistance-proof compounds will maintain their effectiveness indefinitely.

SUMMARY AND CONCLUSIONS

The toxicological test method developed for T. urticae, of spraying the mites affixed to cellulose tape on microscope slides, is accurate and gives reproducible results when at least duplicate lots of 50 adult females are treated at each dose. Low post-treatment temperature markedly reduces mortality, and temperatures above 24°C increase it. Mortality is reduced at very high relative humidities. The susceptibility of the adult females decreases with age, with a plateau during the second week. Differences in pre-treatment temperatures do not significantly affect the test results. Percentage mortalities can differ by as much as 30% according to the time of day when a test is conducted, tending to be lower in the early morning and mid afternoon. The addition of olive oil or corn oil to the acetone solvent increases the mortality obtained.

The susceptible and resistant strains of the Niagara stock of T. urticae studied are both essentially pure. The high resistance of the hybrids indicates that the parathion-resistance is almost completely dominant, and the identical results with reciprocal crosses indicate that it is neither sex-linked nor does it involve any maternal effects. The clear 1:1 segregation obtained in the backcrosses with the susceptible strain proves it to be determined by a single principal gene allele. The resistant strain is not handicapped by being less fecund

or heat-hardy than the susceptible strain, and has a slight superiority in egg viability. The resistant NR strain has almost twice as much detoxicative capacity for malathion as the susceptible strain, most of the malathion being hydrolysed at the carboxyester bond. There is no difference between resistant or susceptible strains in the activity of their cholinesterase enzymes nor in their sensitivity to inhibition by malaoxon.

The cross-resistance from parathion to various OP compounds varies over a wide range, from 2-fold cross-resistance to Bidrin and phosphamidon to 1000-fold to dimethoate; cross-resistance is highest to those OP compounds which are most toxic to the susceptible strain, and conversely. There is no cross-resistance to the chlorinated acaricide dicofol, but a slight cross-resistance to the carbamate Temik. Parathion and Bidrin each select for the same level of cross-resistance to phosphamidon, and Bidrin selects for as high a resistance to parathion and as low a resistance to itself as parathion does. Neither the cross-resistance to Bidrin nor the resistance to parathion is increased by further selecting the parathion-resistant NR strain with Bidrin for 10 generations. Cross-resistance is generally less with methyl than with ethyl OP compounds, but there is no consistent difference in the cross-resistance to compounds with methyl or ethyl groups. There does not appear to be any consistent difference in the degree of cross-resistance to compounds with the phosphate, phosphorothioate or phosphorodithioate groups. The carbomethoxy group and most compounds with the phosphorodithioate group appears to be associated with high toxicity to the susceptible Niagara strain, whereas the carbamoyl group with or

or heat-hardy than the susceptible strain, and has a slight superiority in egg viability. The resistant NR strain has almost twice as much detoxicative capacity for malathion as the susceptible strain, most of the malathion being hydrolysed at the carboxyester bond. There is no difference between resistant or susceptible strains in the activity of their cholinesterase enzymes nor in their sensitivity to inhibition by malaoxon.

The cross-resistance from parathion to various OP compounds varies over a wide range, from 2-fold cross-resistance to Bidrin and phosphamidon to 1000-fold to dimethoate; cross-resistance is highest to those OP compounds which are most toxic to the susceptible strain, and conversely. There is no cross-resistance to the chlorinated acaricide dicofol, but a slight cross-resistance to the carbamate Temik. Parathion and Bidrin each select for the same level of cross-resistance to phosphamidon, and Bidrin selects for as high a resistance to parathion and as low a resistance to itself as parathion does. Neither the cross-resistance to Bidrin nor the resistance to parathion is increased by further selecting the parathion-resistant NR strain with Bidrin for 10 generations. Cross-resistance is generally less with methyl than with ethyl OP compounds, but there is no consistent difference in the cross-resistance to compounds with methyl or ethyl groups. There does not appear to be any consistent difference in the degree of cross-resistance to compounds with the phosphate, phosphorothioate or phosphorodithioate groups. The carbomethoxy group and most compounds with the phosphorodithioate group appears to be associated with high toxicity to the susceptible Niagara strain, whereas the carbamoyl group with or

without a phosphate group appears to be associated with low levels of cross-resistance in the parathion-selected strain.

REFERENCES

Allen, W. W., A. K. Ota, and R. D. Gehring. 1964. The effectiveness of various pesticides against resistant two-spotted spider mites on greenhouse roses. J. econ. Ent. 57: 187-192.

Andres, L. A., and T. Prout. 1960. Selection response and genetics of parathion resistance in the Pacific spider mite, Tetranychus pacificus. J. econ. Ent. 53: 626-630.

Andres, L. A., and H. T. Reynolds. 1958. Laboratory determination of organophosphorus insecticide resistance in three species of Tetranychus on cotton. J. econ. Ent. 51: 285-287.

Armstrong, T., G. G. Dustan, and R. S. Downing. 1954. A comparative study of three acaricides. Annu. Rep. ent. Soc. Ont. 85: 5-17.

Asperen, K. van. 1964. Biochemistry and genetics of esterases in house-flies (Musca domestica) with special reference to the development of resistance to organophosphorus compounds. Ent. exp. & appl. 7: 205-214.

Asperen, K. van, and F. J. Oppenoorth. 1960. The interaction between organophosphorus insecticides and esterases in homogenates of organophosphate susceptible and resistant houseflies. Ent. exp. & appl. 3: 68-83.

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. econ. Ent. 18: 265-267.

Bigley, W. S., and F. W. Plapp Jr. 1960. Cholinesterase and aliesterase activity in organophosphorus susceptible and resistant houseflies. Ann. ent. Soc. Amer. 57: 360-363.

Bliss, C. 1952. The statistics of bioassay. Academic Press, New York.

Bravenboer, L. 1959. The chemical and biological control of the glasshouse red spider. Dissertation, Landbouwhogeschool, Wageningen.

Brown, A. W. A. 1958. Insecticide resistance in arthropods. Wld. Hlth. Organ. Monograph Series No. 38. 240 pp.

Brown, A. W. A. 1960a. Mechanisms of resistance against insecticides. Ann. Rev. Ent. 5: 301-319.

Brown, A. W. A. 1960b. The resistance problem, vector control and WHO. Misc. Publ. ent. Soc. Amer. 2: 59-67.

Brown, A. W. A. 1961a. The challenge of insecticide resistance. Bull. ent. Soc. Amer. 7: 6-19.

Brown, A. W. A. 1961b. Negatively-correlated insecticides - a possible countermeasure for insecticide resistance. Pest Control Mag. 29(1): 24-26.

Brown, A. W. A. 1963a. Meeting the resistance problem: vector control prospects in the light of present knowledge of insecticide resistance. Bull. Wld. Hlth. Organ. 29 Suppl.: 41-50.

Brown, A. W. A. 1963b. -Chemical injuries. In Insect pathology, an advanced treatise. ed. E. A. Steinhaus, Academic Press, New York, 1: 65-131.

Brown, A. W. A. 1963c. Insect resistance. Farm Chem. 126(10): 22-24, 26, 28; (11) 24-25, 28; (12) 17-19, 46 and 127 (1): 58-60.

Brown, A. W. A. 1967. Pest resistance to pesticides. In Treatise on pesticides. ed. R. H. White-Stevens, Chap. 12, Vol. 2. Marcel Dekker, New York. In press.

Busvine, J. R. 1957. A critical review of the techniques for testing insecticides. The Eastern Press Ltd., London and Reading, England. 208 pp.

Busvine, J. R. 1960. The genetics of insecticide resistance. UNESCO Middle East Science Co-operation Office publication. 8 pp.

Chefurka, W. 1967. Mimeographed paper. Can. Dept. Agr. Ottawa.

Cole, C. L., and P. L. Adkisson. 1964. Daily rhythm in the susceptibility of an insect to a toxic agent. Science 144: 1148-1149.

Crow, J. F. 1957. Genetics of insect resistance to chemicals. Ann. Rev. Ent. 2: 227-246.

Cutright, C. R. 1956. A three-year field study of a mite population resistant to parathion. Ohio Agr. Expt. Sta. Res. Circ. 37, 12 pp.

Das, M., and A. H. Needham. 1961. Effects of time and temperature on toxicity of insecticides to insects. III. Tests of seven poisons in the range 10-28°C. Ann. appl. Biol. 49: 267-289.

Dauterman, W. C., and K. N. Mehrotra. 1963. The N-alkyl group specificity of cholinesterase from the housefly, Musca domestica L., and the two-spotted spider mite, Tetranychus telarius L. J. Insect Physiol. 9: 257-263.

Davidson, G. 1960. The importance of the discriminating dosage in the determination of insecticide resistance in anopheline mosquitoes. Misc. Publ. ent. Soc. Amer. 1: 93-94.

Dittrich, V. 1961. Populationsgenetische Untersuchungen an normalen und phosphorsäureester-resistenten Stämmen von Tetranychus urticae. Z. angew. Ent. 48: 34-57.

Dittrich, V. 1962. A comparative study of toxicological test methods on a population of the two-spotted spider mite (Tetranychus telarius). J. econ. Ent. 55: 644-648.

Dittrich, V. 1963a. A recessive factor for organophosphorus-resistance in populations of the two-spotted spider mite, Tetranychus telarius. J. econ. Ent. 56: 182-184.

Dittrich, V. 1963b. Genetics and dynamics of organophosphate resistance in strains of Tetranychus urticae. Adv. Acarol. 1: 238-247.

Dittrich, V. 1966. N-(2-Methyl-4-chlorophenyl)-N',N'-dimethyl-formamidine (C-8514/Schering 36268) evaluated as an acaricide. J. econ. Ent. 59: 889-893.

Farnham, A. W., K. A. Lord, and R. M. Sawicki. 1965. Study of some of the mechanisms connected with resistance to diazinon and diazoxon in a diazinon-resistant strain of houseflies. J. Insect Physiol. 11: 1475-1488.

Finney, D. J. 1952. Probit Analysis. Cambridge University Press.

Fisher, R. W. 1967. Diel periodicity in sensitivity of Tetranychus urticae (Acarina: Tetranychidae) to dicofol. Can. Ent. 99: 281-284.

Fisher, R. W., and I. I. C. Hansell. 1964. Effect of pre- and post-treatment temperatures, age of deposit, and repellency on toxicity of kelthane to the two-spotted mite, Tetranychus telarius (L.) (Acarina: Tetranychidae) Can. Ent. 96: 1307-1312.

Fjelddalen, J. and C. Stenseth. 1962. Kjemikalieresistens hos veksthusspinnmidd (Tetranychus urticae Koch) i Norge. Saertrykk av ((Forskning og forsok i landbruket)). 13: 267-283.

Foott, W. H. 1960. A strain of the European red mite, Panonychus ulmi (Koch) (Acarina: Tetranychidae), resistant to ovex in Southwestern Ontario. Can. J. Plant Sci. 40: 542-545.

Franco, M. G., and F. J. Oppenoorth. 1962. Genetical experiments on the gene for low aliesterase activity and organophosphate resistance in Musca domestica L. Ent. exp. & appl. 5: 119-123.

Garman, P. 1950. Parathion resistant red spiders. J. econ. Ent. 43: 53-56.

Georghiou, G. P. 1965. Genetic studies on insecticide resistance. Adv. Pest Control Res. 6: 171-230.

Georghiou, G. P., and W. R. Bowen. 1966. An analysis of housefly resistance to insecticides in California. J. econ. Ent. 59: 204-214.

Glass, E. H. 1960. Current status of pesticide resistance in insects and mites attacking deciduous orchard crops. Misc. Publ. ent. Soc. Amer. 2: 17-25.

Grebel'sku, S. G., and V. N. Bychenkova. 1962. The insecticidal action of B.H.C. on insects as a function of their physiological condition. Trudy Vostoch. Sibirsk. Fel. Sibirsk. Otdel. Akad. Nauk. SSSR 36: 50-56. Referat Zhur., 1962. No. 62b 211 (Translation).

Hamon, J., and J. Mouchet. 1961. La mesure de la sensibilite des insects aux insecticides: Principes et facteurs de variation. Bull. Soc. entomol. de France. 66: 172-188.

Hansen, C. O. 1958. Cross-resistance induction in the two-spotted mite Tetranychus telarius (L.) Dissertation, Cornell University.

Hansen, C. O., J. A. Naegele, and H. E. Everett. 1963. Cross resistant patterns in the two-spotted spider mite. Adv. Acarol. 1: 257-275.

Harris, C. R., G. F. Manson and J. H. Mazurek. 1962. Development of insecticidal resistance by soil insects in Canada. J. econ. Ent. 55: 777-780.

Harrison, R. A., and Smith, A. G. 1961. Effect of relative humidity on hatching and on toxicity of ovicides to eggs of the red spider mite, Tetranychus telarius (L.) (Acarina: Tetranychidae). Nature (London), 186: 409.

Helle, W. 1959. The occurrence of resistance to organic phosphate compounds in the two-spotted spider mite at Aalsmeer. Tijdschr. Pl. ziekten. 65: 107-115.

Helle, W. 1962. Genetics of resistance to organophosphorus compounds and its relation to diapause in Tetranychus urticae Koch. Dissertation, University of Amsterdam.

Helle, W. 1965a. Resistance in the Acarina: mites. Adv. Acarol. II: 71-93.

Helle, W. 1965b. Expectations of delay of resistance in Tetranychus urticae by rotational sprays. Seventeenth Int'l. Symp. on Phytopharmacy and Phytiatry, 4th May. Meded. LandbHogesch. OpzoekStns Gent 30(3): 1397-1399.

Helle, W. 1965c. Inbreeding depression in an arrhenotokous mite (Tetranychus urticae Koch). Ent. exp. & appl. 8: 299-304.

Henneberry, T. J. 1960. The effect of plant nutrition on the fecundity and susceptibility to malathion of two strains of two-spotted spider mite. Proc. XI inter. Congr. Ent. 2: 49-53.

Herne, D. H. C. 1957. A study of the effects of DDT on reproduction and development of the two-spotted spider mite, Tetranychus telarius (L.). Dissertation, University of

Toronto.

Herne, D. C. 1960. Combinations of acaricides with complementary activity. J. econ. Ent. 53: 967-968.

Herne, D. C. 1961a. European red mite resistance to Kelthane and Guthion. C.D.A. Insecticide Newsletter 10: 5-6.

Herne, D. C. 1961b. Control of parathion-resistant European red mite on peach and plum. C.D.A. Insecticide Newsletter 10: 69-71.

Herne, D. C. 1962. Control of insects and mites. In Res. Rept. 1958-1962, Res. Lab. C.D.A. Vineland Station, Ont. pp. 8-10.

Herne, D. C. 1967. An accurate thermistor psychrometer for small containers. J. econ. Ent. 60: 895.

Herne, D. H. C. and W. L. Putman. 1966. Toxicity of some pesticides to predacious arthropods in Ontario peach orchards. Can. Ent. 98: 936-942.

Hestrin, S. 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. J. biol. Chem. 180: 249-261.

Hill, C. H. 1965. Evaluation of current acaricide programs on apple in Virginia. Paper No. 52. Eastern Branch Meeting, Ent. Soc. Amer. Nov. 1965.

Hoskins, W. M. 1963. Criteria and meaning of tests with the WHO adult mosquito kits. (Unpublished working document WHO/Vector Control/29, WHO/Mal/390.8 pp.).

Hoyer, R. F., F. W. Plapp, and R. D. Orchard. 1965. Linkage relationships of several insecticide resistance factors in the house fly. Ent. exp. & appl. 8: 65-73.

Jacob, F., and J. Monod. 1961. Genetic regulating mechanisms in the synthesis of proteins. J. mol. Biol. 3: 318-356.

Jarczyk, H. J. 1966. The influence of esterases in insects on the degradation of organophosphates of the ^{R} E 605 series. Pflanzenschutz-Nachrichten. (English Edition). 19: 1-34.

Jeppson, L. R. 1960. Resistance of mites attacking citrus. Misc. Publ. ent. Soc. Amer. 2: 13-16.

Jeppson, L. R. 1963. Cross resistance patterns in Acarina. Adv. Acarol. 1: 276-282.

Jeppson, L. R. 1965. Citrus mite resistance to control chemicals. Agrichem. West, Oct. pp. 8, 16, 18.

Jeppson, L. R., and M. J. Jesser. 1962. Laboratory studies on resistance of the Pacific spider mite to acaricides. J. econ. Ent. 55: 78-82.

Jeppson, L. R., J. O. Complin, and M. J. Jesser. 1962. Effects of application programs on citrus red mite control and development of resistance to acaricides. J. econ. Ent. 55: 17-22.

Jeppson, L. R., M. J. Jesser, and J. O. Complin. 1958. Resistance of the citrus red mite to organic phosphates in California. J. econ. Ent. 51: 232-233.

Jeppson, L. R., M. J. Jesser, and J. O. Complin. 1964. Toxicity of phosphoramidothioates and phosphoramidates to susceptible

and acaricide-resistant strains of Tetranychus pacificus and Panonychus citri. J. econ. Ent. 57: 878-881.

Jeppson, L. R., M. J. Jesser, and J. O. Complin. 1965. Response of susceptible strains of the Pacific spider mite and the citrus red mite to O-isopropyl O-(2,4,5-trichlorophenyl) phosphoramidate or phosphoramidothioate. J. econ. Ent. 58: 466-467.

Jeppson, L. R., M. J. Jesser, and J. O. Complin. 1966. Chemical structure and toxicity of some carbamoyloxy phosphorodithioates to susceptible and organophosphorus resistant strains of mites. J. econ. Ent. 59: 185-187.

Kensler, D. L. 1965. A study of factors affecting the vapor toxicity of DDVP to the two-spotted spider mite, Tetranychus urticae Koch. Dissertation, Rutgers - The State University.

Lee, R. M., and P. Batham. 1966. The activity and organophosphate inhibition of cholinesterase from susceptible and resistant ticks (Acari). Ent. exp. & appl. 9: 13-24.

McEnroe, W.D. 1960. Cholinesterase in the two-spotted spider mite. Bull. ent. Soc. Amer. 6:150.

Mailloux, M., and F. O. Morrison. 1962. The effects of acaricides on the developmental stage of the two-spotted spider mite, Tetranychus telarius. J. econ. Ent. 55: 479-483.

March, R. B. 1958. The chemistry and action of acaricides. Ann. Rev. Ent. 3: 355-376.

March, R. B. 1959. Resistance to organophosphorus insecticides. Misc. Publ. ent. Soc. Amer. 1: 13-19.

Matsumura, F., and A. W. A. Brown. 1961. Biochemistry of malathion resistance in Culex tarsalis. J. econ. Ent. 54: 1176-1185.

Matsumura, F., and A. W. A. Brown. 1963. Studies on carboxyesterase in malathion resistant Culex tarsalis. J. econ. Ent. 56: 381-388.

Matsumura, F., and C. J. Hogendijk. 1964. The enzymatic degradation of parathion in organophosphate-susceptible and -resistant houseflies. Ent. exp. & appl. 7: 179-193.

Matsumura, F., and G. Voss. 1964. Mechanism of malathion and parathion resistance in the two-spotted spider mite, Tetranychus urticae. J. econ. Ent. 57: 911-917.

Matsumura, F., and G. Voss. 1965. Properties of partially purified malathion carboxyesterase of the two-spotted spider mite. J. Insect Physiol. II: 147-160.

Mehrotra, K. N. 1961. The occurrence of acetylcholine in the two-spotted spider mite. J. Insect Physiol. 6: 180-184.

Mehrotra, K. N. 1962. Malic dehydrogenase in the two-spotted spider mite, Tetranychus telarius (L.). Can. J. Biochem. Physiol. 40: 1529-1533.

Menn, J. J., and D. G. Stoffey. 1963. Chemical structure and acaricidal activity of certain carbamate phosphate esters. Adv. Acarol. 1: 142-150.

Milani, R. 1960. Genetic studies on insecticide-resistant insects. Misc. Publ. ent. Soc. Amer. 2: 75-83.

Oppenoorth, F. J. 1965. Biochemical genetics of insecticide resistance. Ann. Rev. Ent. 10: 185-206.

Oppenoorth, F. J., and K. van Asperen. 1960. Allelic genes in the housefly produces modified enzymes that cause organophosphate resistance. Science 132: 298-299.

Pal, R., and R. L. Kalra. 1966. Insecticide resistance, a review of developments in 1960-1963. Pans, Sec. A, 12: 84-86.

Perry, A. S. 1964. The physiology of insecticide resistance by insects, pp. 285-378. In The physiology of insecta. Vol. 3. ed. M. Rockstein. Academic Press, New York.

Polcik, B., J. W. Nowosielski, and J. A. Naegele. 1964. Daily rhythm of the two-spotted mite, Tetranychus urticae to DDVP. Science 145: 405-406.

Putman, W. L., and D. C. Herne. 1959. Gross effects of some pesticides on populations of phytophagous mites in Ontario peach orchards and their economic implications. Can. Ent. 91: 567-579.

Quarterman, K. D. 1960. Test methods for establishing levels of susceptibility and detecting the development of resistance in insects of public health importance. Misc. Publ. ent. Soc. Amer. 2: 95-102.

Reynolds, H. T. 1960. Establishing levels of insecticide resistance with standardized laboratory detection methods in agricultural arthropod pests. Misc. Publ. ent. Soc. Amer. 2:

103-111.

Reynolds, H. T. 1962. Standardized laboratory detection methods for resistance determination in agricultural arthropod pests. Bull. ent. Res. 8: 9-14.

Robbins, W. E., T. L. Hopkins, and A. R. Roth. 1958. Application of the colorimetric whole-blood method to the measurement of bovine red-blood-cell cholinesterase activity. J. econ. Ent. 51: 326-329.

Saba, F. 1961a. Über Entwicklung und Rückgang von Giftresistenz bei Tetranychus urticae Koch und deren Abhängigkeit von der Wirtspflanze. Z. angew. Ent. 48: 265-293.

Saba, F. 1961b. Über die Bildung der Diapauseform bei Tetranychus urticae Koch in Abhängigkeit von Giftresistenz. Ent. exp. & appl. 4: 264-272.

Saba, F. 1962. Kopulationsfrequenz, Giftempfindlichkeit und Heterogenitat von Weibchen und Mannchen bei Tetranychus urticae Koch. Anz. Schädlingsk. 35(9): 141-142.

Schatz, A., E.B. Schalscha, and V. Schatz. 1964. Soil organic matter as a natural chelating material. Part 2: The occurrence and importance of paradoxical concentration effects in biological systems. Compost Science 5: 26-31.

Schulten, G. G. M. 1966. Genetics of resistance to parathion and demeton-S-methyl in Tetranychus urticae Koch (Acarina). Genetica 37: 207-217.

Smissaert, H. R. 1964. Cholinesterase inhibition in spider mites susceptible and resistant to organophosphate. Science 143:

129-131.

Smissaert, H. R. 1965. Esterases in spider mites hydrolysing ∝-naphthylacetate. Nature (London), 205: 158-160.

Smith, F. F. 1960. Resistance of greenhouse spider mites to acaricides. Misc. Pub. Ent. Soc. Am. 2(1): 5-12.

Storherr, R. W., M. E. Getz, R. R. Watts, S. J. Friedman, F. Erwin, L. Giufrida, and F. Ives. 1964. Identification and analyses of five organophosphate pesticides: Recoveries from crops fortified at different levels. J. Assoc. offic. agr. Chem. 47: 1087-1093.

Taylor, E. A., and F. F. Smith. 1956. Transmission of resistance between strains of two-spotted spider mites. J. econ. Ent. 49: 858-859.

Tew, R. P., and A. H. M. Kirby. 1953. Agricultural insecticides Rep. Prog. appl. Chem. 38: 632-640.

Triolo, A. J., and J. M. Coon. 1966. Toxicological interactions of chlorinated hydrocarbon and organophosphate insecticides.. J. agr. Food Chem. 14: 549-555.

Tsukamoto, M. 1963. The log dosage-probit mortality curve in genetic researches of insect resistance to insecticides. Botyu-Kagaku 28: 91-98.

Unterstenhöfer, G. 1960. On the present level of resistance to insecticides and acaricides. Höfchen-Briefe (English Edition). 13: 141-150.

Unterstenhöfer, G. 1961. Development of spider mite resistance to acaricides. Höfchen-Briefe (English Edition). 14: 1-6.

Voss, G. 1960. Esterasen bei der Spinnmilbe, Tetranychus urticae Koch. Naturwissenschaften 47: 400-401.

Voss, G. Von. 1961. Ein neues Akarizid-Austestungsverfahren für Spinnmilben. Anz. Schadlingsk. 34: 76-77.

Voss, K. G. 1962. Physiologische und toxikologische Untersuchungen an der Spinnmilbe Tetranychus urticae Koch unter besonderer Berücksichtigung eines phosphorsaureesterresistenten Stammes. Dissertation, University Würzburg.

Voss, G., and F. Matsumura. 1964. Resistance to organophosphorus compounds in the two-spotted spider mite: Two different mechanisms of resistance. Nature (London), 202: 319-320.

Voss, G., and F. Matsumura. 1965. Biochemical studies on a modified and normal cholinesterase found in the Leverkusen strains of the two-spotted spider mite Tetranychus urticae Can. J. Biochem. 43: 63-72.

Voss, G., W. C. Dauterman, and F. Matsumura. 1964. Relation between toxicity of malathion analog and organophosphorus resistance in the two-spotted spider mite. J. econ. Ent. 57: 808-811.

Watson, D. L. 1956. Some genetic aspects of resistance to parathion in the two-spotted spider mite (Tetranychus telarius L.). Dissertation, Cornell University.

Watson, D. L., and J. A. Naegele. 1960. The influence of selection pressure on the development of resistance in populations of Tetranychus telarius (L.). J. econ. Ent. 53: 80-84.

Weiden, M. H. J., H. H. Moorefield, and L. K. Payne. 1965. O-(methylcarbamoyl) oximes: A new class of carbamate insecticides-acaricides. J. econ. Ent. 58: 154.

Whitnall, A. B. M. 1962. Studies on the control of orchard mites. S. Afr. J. Sci. 58: 101-108.

Winston, P. W., and D. H. Bates. 1964. Saturated solutions for the control of humidity in biological research. Ecology 41: 232-237.

Winteringham, F. P. W. 1962. Action and inaction of insecticides. J. roy. Soc. Arts. 110: 719-740.

Wood, G. C. 1952. Insecticide resistance in the two-spotted spider mite (Tetranychus bimaculatus Harvey). Dissertation, Cornell University.

Yamamoto, S., and A. Nishida. 1961. Studies on the pesticide resistance of citrus red mite, Panonychus citri McGregor. I. On the organophosphorus resistant mite and its cholinesterase activity. Takamine Kenkyusho Nempo. 13: 237-240.

Zon, A. Q. van, and W. Helle. 1966. A search for linkage between genes for albinism and parathion resistance in Tetranychus pacificus McGregor. Genetica. 37: 181-185.

APPENDIX 1

Table 1 shows the variation in the dosage-mortality experiments for the different strains and crosses used in the inheritance of resistance study. Tables 2 to 9 show the variation in the dosage-mortality experiments with the different test compounds for the 5 Niagara strains of T. urticae used in this investigation.

Table 1. Toxicity of parathion to the susceptible and resistant Niagara strains of T. urticae, and the F_1 offspring of the reciprocal crosses, and I x R cross.

	Strain or Cross				
	NS	NR	R x S	S x R	I x R
Slope	4.52	3.49	4.05	3.08	3.48
S.E.	0.320	0.411	0.446	0.666	0.328
LD_{50}	0.050	3.19	3.65	3.43	2.47
95% f.l.	0.043, 0.059	2.59, 3.76	3.25, 4.05	1.65, 5.07	2.18, 2.76
LD_{95}	0.115	9.44	9.31	11.74	7.34
95% f.l.	0.103, 0.128	7.01, 17.37	7.85, 11.95	7.01, 128.65	6.18, 9.28

Data based on values from 2 to 4 experiments per strain or cross (100 to 200 mites per point)

Table 2. Toxicity of parathion to the susceptible NS and resistant NR Niagara strains of *T. urticae* over a two-year period.

	Strain and year tested			
	NS (1964)	NS (1965)	NR (1964)	NR (1965)
Slope	3.24	2.67	3.49	3.64
S.E.	0.207	0.231	0.411	0.186
LD_{50}	0.037	0.025	3.19	3.20
95% f.l.	0.035, 0.040	0.028, 0.022	2.59, 3.76	3.05, 3.36
LD_{95}	0.120	0.102	9.44	9.06
95% f.l.	0.107, 0.140	0.085, 0.131	7.01, 17.37	8.19, 10.23

Values based on data from a minimum of 4 experiments per strain per year i.e. 200 mites per point.

Table 3. Toxicity of demeton and fenthion to the susceptible and resistant NR Niagara strains of T. urticae.

	demeton		fenthion	
	Strain		Strain	
	NS_{21}	NR	NS	NR
Slope	2.22	2.69	2.49	3.38
S.E.	0.171	0.124	0.165	0.242
LD_{50}	0.008	0.513	0.041	1.88
95% f.l.	0.007, 0.010	0.466, 0.564	0.037, 0.045	1.73, 2.05
LD_{95}	0.045	2.10	0.187	5.77
95% f.l.	0.033, 0.071	1.81, 2.50	0.151, 0.245	4.92, 7.09

Values based on data from 2 to 3 experiments per strain per compound, i.e. 100 to 150 mites per point.

Table 4. Toxicity of malathion and ethion to the susceptible and resistant NR Niagara strains of T. urticae.

	malathion		ethion	
	Strain		Strain	
	NS	NR	NS 21	NR
Slope	2.54	6.55	3.51	3.98
S.E.	0.379	0.510	0.48	0.27
LD_{50}	0.095	2.90	0.0096	3.52
95% f.l.	0.059, 0.129	2.77, 3.03	0.0062, 0.013	3.32, 3.73
LD_{95}	0.447	5.17	0.028	9.12
95% f.l.	0.275, 1.633	4.73, 5.82	0.019, 0.116	8.09, 10.64

Values based on data from 3 to 4 experiments per strain per compound, i.e. 150 to 200 mites per point.

Table 5. Toxicity of dimethoate and mevinphos to the susceptible NS_{21}, and resistant NR Niagara strains of T. urticae.

	dimethoate		mevinphos	
	NS_{21}	NR	NS_{21}	NR
Slope	3.54	7.40	6.07	5.39
S.E.	0.319	0.471	0.554	0.549
LD_{50}	0.004	4.02	0.0038	0.570
95% f.l.	0.0035, 0.0045	3.87, 4.15	0.0035, 0.0042	0.525, 0.617
LD_{95}	0.011	6.69	0.0071	1.15
95% f.l.	0.009, 0.015	6.22, 7.39	0.0060, 0.0090	1.01, 1.38

Values based on data from 2 to 4 experiments per strain per compound, i.e. 100 to 200 mites per point.

Table 6. Relative toxicity of Bidrin to the susceptible and OP-resistant Niagara strains of *T. urticae*.

	Strains				
	NS	NS_{21}	NR	NBR	NRBR
Slope	2.76	3.03	3.61	3.54	3.44
S.E.	0.174	0.213	0.265	0.435	0.259
LD_{50}	0.026	0.030	0.057	0.075	0.061
95% f.l.	0.023, 0.028	0.029, 0.033	0.052, 0.062	0.059, 0.089	0.056, 0.067
LD_{95}	0.103	0.105	0.164	0.220	0.184
95% f.l.	0.093, 0.116	0.091, 0.126	0.146 0.189	0.169, 0.355	0.161, 0.218

Data based on values from 3 to 5 experiments per strain, i.e. 150 to 250 mites per point.

Table 7. Relative toxicity of phosphamidon to the susceptible and OP-resistant Niagara strains of T. urticae.

	Strain			
	NS	NS_{21}	NR[a]	NBR[b]
Slope	3.24	2.98	3.02	2.95
S.E.	0.194	0.171	0.190	0.279
LD_{50}	0.073	0.075	0.130	0.134
95% f.l.	0.068, 0.078	0.069, 0.081	0.120, 0.141	0.112, 0.156
LD_{95}	0.236	0.272	0.457	0.483
95% f.l.	0.209, 0.272	0.239, 0.319	0.395, 0.547	0.370, 0.729

[a]NR, parathion-selected strain.

[b]NBR Bidrin-selected strain.

Values based on data from 3 experiments per strain, i.e. 150 mites per point.

Table 8. Toxicity of parathion to the organophosphorus-resistant Niagara strains of T. urticae.

	Strain		
	NR[a]	NBR[b]	NRBR[c]
Slope	3.56	4.29	3.58
S.E.	0.429	0.367	0.235
LD_{50}	1.93	1.97	1.92
95% f.l.	1.58, 2.33	1.82, 2.11	1.78, 2.06
LD_{95}	5.61	4.76	5.52
95% f.l.	4.18, 9.66	4.19, 5.63	4.86, 6.47

[a]NR, selected with parathion

[b]NBR, selected with Bidrin

[c]NRBR, NR strain selected with Bidrin for 10 generations

Data based on a minimum of 3 replicated experiments, i.e. 150 mites per point.

Table 9. Toxicity of dicofol to the susceptible NS_{21} and resistant NR, Niagara strains of T. urticae.

	NS_{21}[a]	NR[b]
Slope	5.31	5.70
S.E.	0.625	0.468
LD_{50}	0.032	0.032
95% f.l.	0.027, 0.036	0.029, 0.033
LD_{95}	0.064	0.062
95% f.l.	0.052, 0.059	0.055, 0.070

[ab]Values based on data from 3 experiments and 2 experiments respectively.

APPENDIX 2

Figures 1 and 2 show the dosage-mortality relationships to ethion and dimethoate for substrains reared from individual females, found to be homozygous susceptible by the male discriminating-dose method, when tested with up to 19 closely-spaced dosages. Figure 3 shows the dosage-mortality relationship to parathion for the partially-resistant parathion-selected I strain adult females tested in 1964 and 7 months later in 1965.

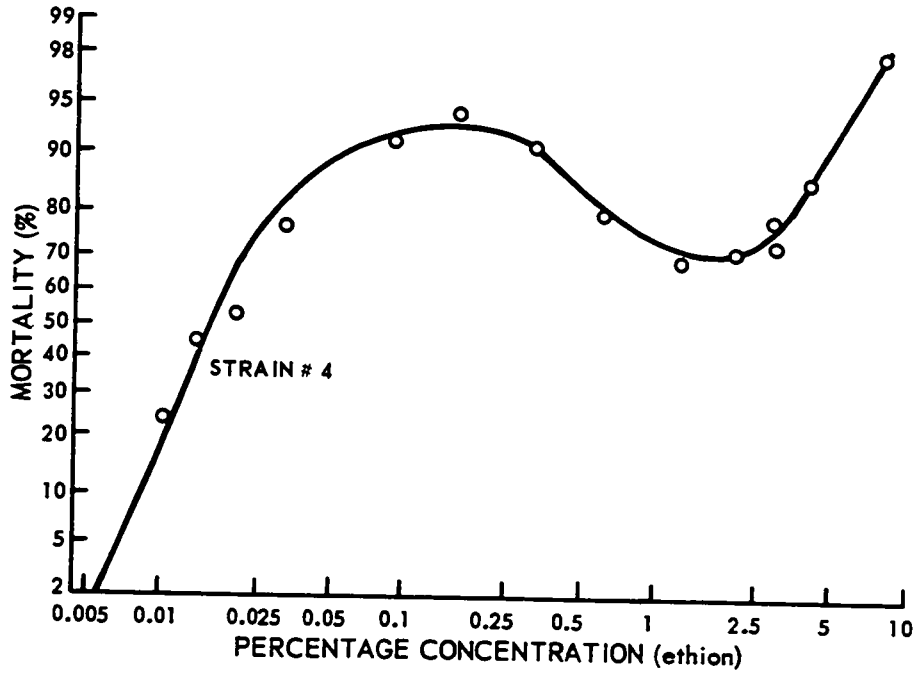


Fig. 1. Dosage-mortality relationship to ethion for substrain # 4 reared from one NS strain female chosen by the male discriminating-dose method.

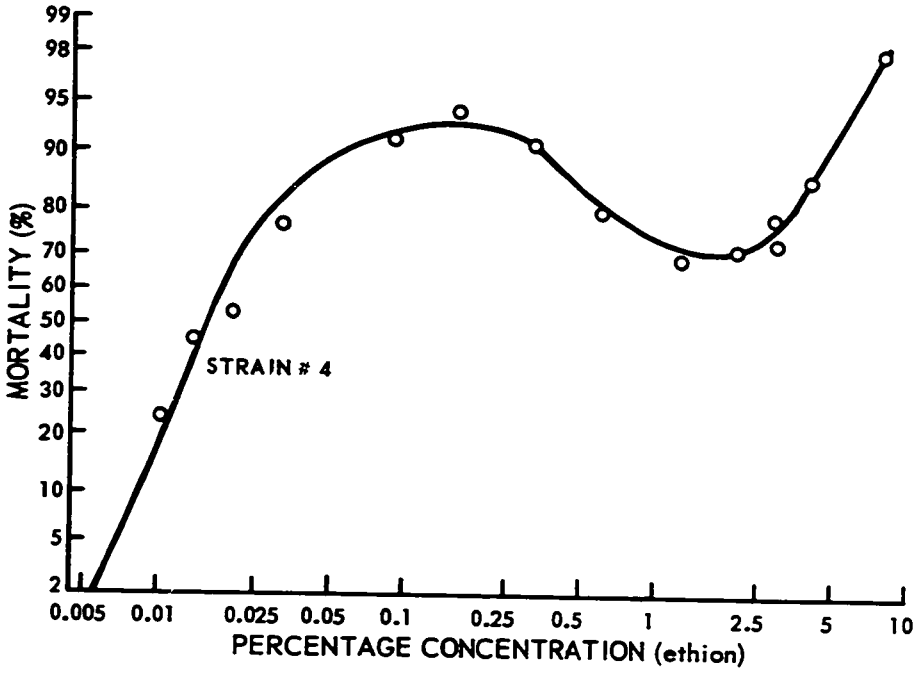


Fig. 1. Dosage-mortality relationship to ethion for substrain # 4 reared from one NS strain female chosen by the male discriminating-dose method.

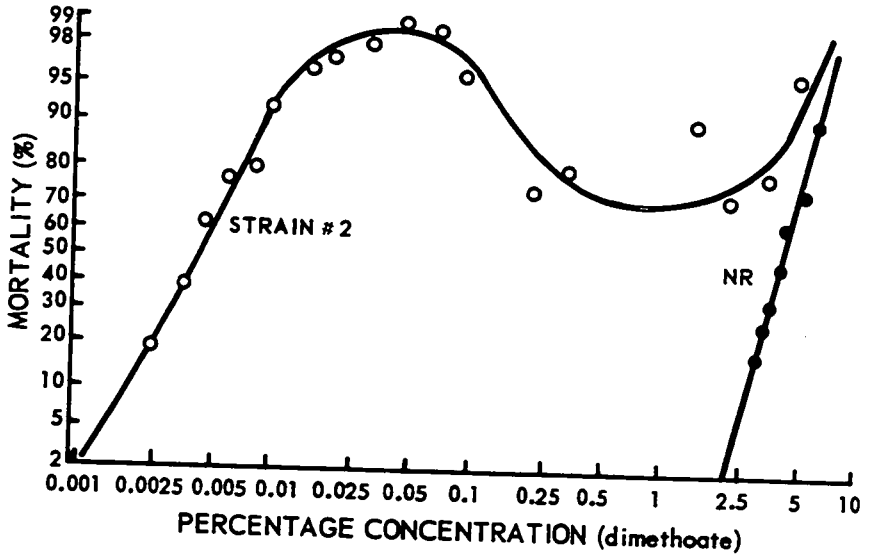


Fig. 2. Dosage-mortality relationship to dimethoate for substrain # 2 reared from one NS strain female chosen by the male discriminating-dose method, for comparison with that of the NR strain.

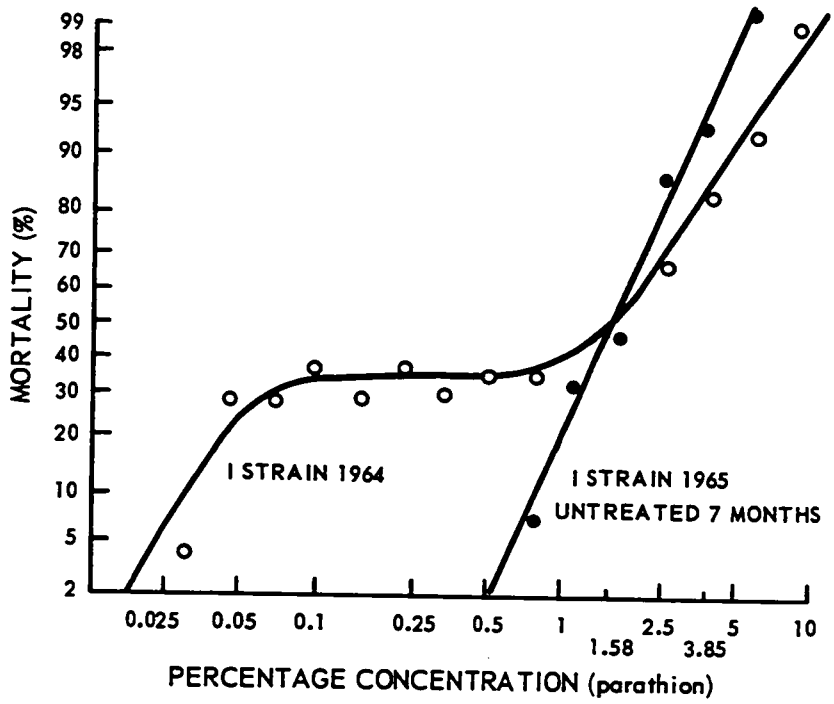


Fig. 3. Dosage-mortality relationship to parathion for the I strain tested in 1964 and in 1965.